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Pestilence, persistence and pathogenicity: infection strategies of *Bartonella*

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Abstract

It has been nearly two decades since the discovery of *Bartonella* as an agent of bacillary angiomatosis in AIDS patients and persistent bacteremia and 'nonculturable' endocarditis in homeless people. Since that time, the number of *Bartonella* species identified has increased from one to 24, and 10 of these bacteria are associated with human disease. Although *Bartonella* is the only genus that infects human erythrocytes and triggers pathological angiogenesis in the vascular bed, the group remains understudied compared with most other bacterial pathogens. Numerous questions regarding *Bartonella*'s molecular pathogenesis and epidemiology remain unanswered. Virtually every mammal harbors one or more *Bartonella* species and their transmission typically involves a hematophagous arthropod vector. However, many details regarding epidemiology and the public health threat imposed by these animal reservoirs is unclear. A handful of studies have shown that bartonellae are highly-adapted pathogens whose parasitic strategy has evolved to cause persistent infections of the host. To this end, virulence attributes of *Bartonella* include the subversion of host cells with effector molecules delivered via a type IV secretion system, induction of pathological angiogenesis through various means, including inhibition of apoptosis and activation of hypoxia-inducing factor 1, use of afimbrial adhesins that are orthologs of *Yersinia* adhesin A, incorporation of lipopolysaccharides with low endotoxic potency in the outer membrane, and several other virulence factors that help *Bartonella* infect and persist in erythrocytes and endothelial cells of the host circulatory system.

Keywords

angiogenesis; *Bartonella*; bartonellosis; hemotrophy; infection; virulence

Bartonella bacilliformis

Bartonella are widely regarded in the biomedical community as emerging bacterial agents of infectious disease. In fact, modern-day bartonelloses are preceded by a long history and an incidence that inversely correlates with the human condition. Archaeological evidence suggests that these pathogens have afflicted humans for several millennia. Anthropomorphic pottery

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jugs (huacos) and carvings made by pre-Columbian Indians in endemic regions of South America frequently bear the telltale angiomatous lesions of Oroya fever, termed verruga peruana, a sequela of chronic infection by *Bartonella bacilliformis* (Figure 1). Dissemination of knowledge regarding the dangers of exposure to the pathogen was minimal at this time, since the indigenous people had no written language, and the disease was geographically restricted to the high Andes. In fact, the disease potential of *B. bacilliformis* would not be fully appreciated for several centuries. In 1540, Spanish conquistadores led by Pizarro were likely the first foreigners to experience an epidemic of acute febrile illness followed by 'warts full of blood' in the survivors [1]. Later, during the building of the highest portion of the Central Railroad of Peru, Oroya fever resulted in 8000 fatalities in only 4 years (1869–1873). It was not until the late 19th century that a Peruvian medical student, Daniel Alcides de Carrión, demonstrated that verruga peruana lesions could be used as an inoculum to elicit the more acute febrile syndrome (Oroya fever). Tragically, the experiment was done using his own body as a model, and he died of Oroya fever at 28 years of age. To this day, the acute and chronic manifestations of *B. bacilliformis* infection are referred to as Carrión's disease, and Carrión is a national hero in Peru. Several lines of evidence suggest that *B. bacilliformis* is the sole representative of an 'ancestral' lineage from which other host-adapted 'modern' *Bartonella* species have evolved (see 'Erythrocyte Parasitism').

Bartonella quintana

The second major historical event involving *Bartonella* coincided with World Wars I and II. Trench warfare, POW facilities and concentration camps were ideal scenarios for the transmission of louse-borne trench fever, caused by *Bartonella quintana* (formerly *Rochliamaea* or *Rickettsia quintana*). In fact, trench fever epidemics were commonplace during World War I and its morbidity for troops was only surpassed by influenza; a major pandemic at this time. For almost five decades following World War II, trench fever was observed only sporadically in developing nations. Then, in the mid-1990s, *B. quintana* was recognized as a re-emerging agent of 'urban trench fever', afflicting a significant portion of homeless inner-city populations throughout the world [2]. Interestingly, homeless people share many of the same risk factors for trench fever as troops or prisoners afflicted during war, including malnutrition, poor hygiene, louse infestation, alcohol abuse and an immunocompromised state.

Bartonella henselae

The third major historical event involving *Bartonella*, and one that helped clarify the genus' phylogenetic relatedness to other α-proteobacteria, including rhizobia and *Brucella*, was the increase in cases of AIDS-related complex in the late 1980s. In an interesting clinical detective story, both *Bartonella henselae* and *B. quintana* were shown by several labs to be frequent secondary pathogens of AIDS patients [3,4]. In a majority of these cases, bartonellosis manifested as one or a combination of vascular pathologies, including bacillary angiomatosis (BA) of the skin (similar to verrugas), bacillary peliosis of the liver and spleen, nonculturable endocarditis and chronic bacteremia.

Currently, there are two dozen recognized *Bartonella* species, 10 of which are associated with human disease [5]. Nevertheless, the majority of reported human bartonelloses are caused by three species: *B. bacilliformis*, *B. quintana* and *B. henselae*, and these pathogens are the focus of this article.

Transmission & colonization of the human host

Bartonella are transmitted to humans via two routes: hematophagous insects, such as phlebotamine sand flies (*B. bacilliformis*), human body lice (*B. quintana*) and cat fleas (*B. henselae*); and animal scratches and bites (*B. henselae*). In certain instances, the distinction

between vector and reservoir is blurred by a lifelong infection of the vector, as seen in human body lice infected with *B. quintana* [6] and perhaps in sand flies infected with *B. bacilliformis* [7]. The role of ticks in transmission to humans is suspected and supported by indirect evidence [8]. It is also important to note that unlike *B. quintana* and *B. bacilliformis*, *B. henselae* also utilizes nonhuman reservoirs (e.g., cats and dogs [8]).

When *Bartonella* gains access to the human circulatory system, it disseminates from the point of inoculation by passive means. Motility conferred by lophotrichous flagella (*B. bacilliformis*) may contribute to spreading, but the importance of this appendage in host colonization has not been conclusively demonstrated. What is clear is that *Bartonella* can colonize secondary foci at considerable distances from the primary site of infection, and there is a preference for highly vascularized tissues like heart valves, liver and spleen (*B. quintana* and *B. henselae*), or cooler areas of the body, such as the vascular bed of the skin (*B. bacilliformis*). It is in the context of these particular locations that *Bartonella* interacts with its two major host cell types – erythrocytes and endothelial cells.

Erythrocyte parasitism

One of the most fascinating aspects of a *Bartonella* infection is the intracellular parasitism of erythrocytes, a practice termed hemotrophy. Although bacterial pathogens of other vertebrates, such as *Anaplasma*, also employ this parasitic strategy, in this regard, *Bartonella* is unique amongst bacterial pathogens of humans. Of the three major pathogenic *Bartonella*, only *B. henselae* and *B. quintana* establish chronic intraerythrocytic bacteremias. In stark contrast, *B. bacilliformis*' hemotrophy is relatively short-lived and potentially life-threatening, presumably due to splenic culling of infected cells (nearly every erythrocyte in circulation) and the attendant drop in hematocrit (~80% decrease).

Factors that contribute to erythrocyte tropism include flagella-mediated motility, and surface proteins associated with the Trw type IV secretion system (T4SS) (Figure 2). Remarkably, genes for flagella-based motility, and Trw are mutually exclusive; the ‘ancestral’ *Bartonella* lineage, *B. bacilliformis*, is flagellated and lacks Trw, whereas more recently descended lineages, including *B. quintana* and *B. henselae* (‘modern’ *Bartonella*), are nonflagellated but possess Trw (Table 1) [5,9]. Early work showed that *B. bacilliformis*' binding to erythrocytes was absent in nonmotile or killed bacteria [10,11]. Later research showed that if *B. bacilliformis*' motility is impaired by antiflagellin antibodies, bacterial association and invasion of human erythrocytes is significantly decreased *in vitro*, suggesting that motility increases the pathogen's ability to parasitize host erythrocytes [12].

Another factor thought to enhance hemotrophy by *B. bacilliformis* and, possibly, *B. henselae*, is deformation factor or ‘deformin’ (Figure 2) [13]. Deformin is released into the culture medium during *in vitro* growth and can independently cause extensive pitting and invagination of erythrocyte cell membranes. To what extent these membrane changes contribute to *Bartonella*'s colonization of erythrocytes *in vivo* is unknown. Initial analyses showed that deformin was a heat-sensitive protein of 130 kDa (native molecular mass) [14, 15], whereas subsequent research showed that it was a small, hydrophobic molecule of 1400 Da with a high affinity for albumin [16]. Clarification of deformin's biochemical nature, its occurrence in other *Bartonella* species and role in hemotrophy *in vivo* warrant additional research, especially given the marked morphological changes that it can cause in erythrocytes.

More recent work has revealed that the Trw T4SS, with its multiple variant copies of pilin (TrwL) and pilus-associated component (TrwJ), is involved in erythrocyte adhesion in *Bartonella tribocorum* (a rodent pathogen) and is required to establish chronic intraerythrocytic bacteremia [17]. The Trw systems of *B. quintana* and *B. henselae* may play analogous roles in

human infections (Figure 2). Interestingly, the absence of a TrwB (VirD4) ortholog suggests that *Bartonella*'s Trw system has lost the ability to translocate T4SS effector molecules [5]. Instead, the multigenic nature and coexpression of Trw components is thought to provide a means of helping to establish host range by virtue of recognition and binding to cognate erythrocyte receptor(s). The nature of *B. bacilliformis*' erythrocyte adhesin(s) is a mystery, despite its ability to practice hemotrophy without a Trw system.

The nature of erythrocyte ligands(s) participating in *Bartonella*'s adherence remains unclear. Early work by Walker and Winkler showed that *B. bacilliformis* has a binding preference for human erythrocytes over those from other mammals and that the receptor(s) is sensitive to α- or β-glucosidase treatment [11]. Later work revealed that several erythrocyte proteins, including glycophorins A and B, are recognized by *B. bacilliformis* and that the carbohydrate moieties are important for the pathogen's binding [18].

Erythrocyte invasion by *Bartonella* is an intriguing and central event in pathogenesis. Early work with *B. bacilliformis* identified an invasion-associated gene, termed *ialB* (Figure 2), which could confer an erythrocyte-invasive phenotype on laboratory-adapted strains of *Escherichia coli* [19]. Later work showed that *ialB*-knockout mutants of *B. bacilliformis* were severely impaired (~50% reduction) in their ability to invade human erythrocytes *in vitro*, and the phenotype could be restored by *trans*-complementation [20]. Two studies localized the IalB protein to the inner and outer membrane fractions of *B. bacilliformis* and *B. henselae*, respectively [20,21]; a contradiction that warrants clarification. Interestingly, *ialB* expression is responsive to two environmental cues that would likely be encountered by *B. bacilliformis* in the context of the sandfly midgut. For example, *ialB* expression and IalB synthesis are highest under arthropod-like conditions (20°C, pH 5.0) when compared with human-like conditions (37°C, pH 7.2) [22]. These cues could conceivably ‘prime’ the bacterium during its time in the vector, enhancing its virulence for subsequent transmission to humans when the sandfly feeds again in a few days. The mechanism whereby IalB augments erythrocyte invasion by *Bartonella* is unknown but clearly intriguing.

Finally, *Bartonella*'s hemolytic proteins may play a role in hemotrophy (Table 1 & Figure 2). For example, *B. bacilliformis* produces a contact-dependent hemolysin that is maximally expressed during exponential-phase growth [23]. The hemolysin acts independently of deformin and may be responsible for the β-hemolytic phenotype observed with *B. bacilliformis* after several days of growth on blood agar. It is tempting to speculate that contact-dependent hemolysin is utilized by *B. bacilliformis* to escape from vacuoles or host cells during intracellular parasitism. A second group of hemolytic proteins is the cohemolysins. By definition, cohemolysin activity is only apparent in the presence of a β-hemolysin, where it causes a synergistic hemolysis reaction (i.e., a CAMP test reaction). Work by Litwin and Johnson identified a cohemolysin, termed Cfa, of *B. henselae* that is a 180-kDa autotransporter protein with orthologs present in *B. quintana*. The potential virulence function of Cfa is intriguing, especially since its α domain is secreted into the culture medium during *in vitro* growth and the protein has some homology to repeat in toxin (RTX) hemolysins [24]. The virulence function of Cfa, characterization of Cfa orthologs from other *Bartonella* and analysis of other autotransporter proteins of bartonellae, such as *B. henselae*'s acidic repeat protein [25], need further investigation.

Adherence to & invasion of endothelial cells

Bartonella's YadA-like outer membrane proteins (omps) constitute a family of afimbrial adhesins for endothelial cells (ECs). *Bartonella* YadA-like omps are defined as trimeric autotransporter adhesins (TAAs), in reference to their trimeric coiled–coil surface structure, use of type V secretion during export and a conserved role in adherence to host cells [26].

Bartonella TAAs were once thought to be type IV pili, based upon their superficial resemblance to these appendages under high magnification [27]. TAAs are large proteins (up to ~330-kDa monomers) that form four major domains, including a C-terminal β-barrel anchor in the outer membrane (which also serves as a pore during secretion), a highly repetitive and variable coiled–coil stalk domain, and a short, conserved neck region that bridges the stalk to a head domain consisting of larger β-helices [26]. *Bartonella* TAA orthologs include: *Bartonella* adhesin A (BadA) of *B. henselae*, the variably-expressed omps (Vomps) of *B. quintana*, and *Bartonella* repetitive protein A (BrpA) of *B. vinsonii*. To prevent confusion by the disparate nomenclature of the various *Bartonella* orthologs, we will collectively refer to them as TAAs.

B. henselae's TAA (BadA) is essential for binding ECs through its recognition of host cell β1-integrins [28]. In addition, BadA mediates binding to extracellular matrix proteins, such as collagens and fibronectin, the latter of which may serve to bridge the adhesin and β1 integrins [28,29]. This TAA also significantly inhibits phagocytosis by J774 cells *in vitro* as compared with wild-type strains [28]. Recent work has shown that the head of BadA is crucial for binding EC and collagen, whereas the stalk is essential for fibronectin binding [30]. This same study also showed that just the BadA head domain can promote auto-aggregation of *B. henselae* cells, as observed in strains expressing intact BadA.

B. quintana's TAAs (Vomps) are also involved in auto-aggregation of the bacterium and collagen IV binding [31]. Interestingly, differential expression of Vomps by various strains of *B. quintana* does not appear to correlate with the ability of the pathogen to adhere to cocultured macrophage (THP-1) or epithelial (HeLa 229) cells [32]. A recent investigation also showed that a *vomp* null-mutant strain of *B. quintana* was unable to establish bacteremia in the macaque model of infection [33]. However, a bacteremic state could be generated in macaques by utilizing a partial-null mutant containing *vompC* and *vompD* genes. Remarkably, natural mutants for *vompA* and *vompB* arise *in vivo* in chronically infected macaques [31]. Characterizing Vomp receptors on host cells would be an interesting study, as they may be differentially employed to parasitize a variety of tissue types within the host.

Recent work has shown that *Bartonella*'s heme-binding proteins (Hbps, also called Pap31), may represent another set of potential adhesins for ECs and/or extracellular matrix constituents such as fibronectin and heparin [34]. Given the relatedness of Hbps to gonococcal opacity (Opa) proteins, their extremely hydrophobic nature and surface location in bartonellae, an adhesin function is certainly possible and would effectively provide a multifunctional role for the Hbps.

Following adherence to ECs, *Bartonella* likely enters the cell by an actin-dependent process. For example, in *B. bacilliformis*, internalization was shown to involve the actin GTPases, Cdc42, Rho and Rac [35], and in *B. henselae* the process culminates in the formation of blebs containing several internalized bacteria [36]. A noteworthy assemblage has also been observed during EC internalization of *B. henselae*, termed the ‘invasome’. This basket-like structure develops over a comparatively longer time period (24 h) than typical internalization, involves extensive actin reorganization during its formation and provides for internalization of a large aggregate of bartonellae [37].

Recent evidence indicates that *B. henselae* avoids the normal endocytic pathway by utilizing a specialized *Bartonella*-containing vacuole (BCV) that does not acidify or fuse with lysosomes in ECs or is delayed in macrophages [38]. The BCV provides an ideal compartment for bacterial replication at a neutral pH and without the onslaught of innate immune effectors or degradative enzymes stored in the lysosomes. The mechanism whereby endosome maturation is inhibited is unknown, but *Bartonella* obviously plays an active role in the process, as dead bacteria are processed via normal phagolysosome formation. Screening of a mutant library of *B.*

henselae for reduced evasion of the endocytic pathway identified *vapA5* (a hypothetical virulence gene), *hbpD* (encoding HbpD) and *cycA* (encoding an amino acid transporter). Each of these mutations could be complemented in *trans* with the wild-type gene to restore the lysosome-evasion phenotype [38].

Host cell subversion

Type IV secretion systems are elegant macromolecular machines used to deliver bacterial effector molecules into a host cell. T4SS effectors, in turn, subvert the recipient cell to accommodate the pathogen's specific growth requirements and conditions [39]. T4SS are found in a growing list of bacterial pathogens, including *Coxiella burnetii*, *Agrobacterium tumefaciens* and *Helicobacter pylori*, where they provide efficient injection of effector molecules involved in processes, such as inhibiting apoptosis, enhancing tumorigenicity and remodeling of the host cell's actin cytoskeleton [40]. *B. bacilliformis*, the ancestral *Bartonella* lineage, lacks a T4SS, whereas descendent lineages (modern species), such as *B. henselae* and *B. quintana* possess two T4SS, including the VirB/VirD4 (T4SS effector translocation) and Trw (pilin) system (Table 1 & Figure 2) [9]. Acquisition of T4SS operons has undoubtedly allowed certain *Bartonella* species to expand their host range and to subjugate host cells in ways that are not possible in *B. bacilliformis*. In this section, we will focus on the VirB/VirD4 system of *B. quintana* and *B. henselae*, as Trw's role as an adhesin for host erythrocytes was described previously.

The VirB/VirD4 T4SS of *Bartonella* was inadvertently discovered during cloning and characterization of *B. henselae*'s immunodominant 17-kDa protein. The 17-kDa antigen is actually a VirB5 homolog and is encoded in the *virB* operon [41,42]. Subsequent work has shown that the VirB/VirD4 T4SS is primarily involved in establishing EC parasitism [43] and is responsible for some of the EC pathology observed during bartonellosis (Figure 2), including the extensive actin-based cytoskeletal reconfiguration, activation of nuclear factor (NF)-κB leading to a proinflammatory state which fosters chronic inflammation and inhibition of EC apoptosis [40]. T4SS-translocated proteins responsible for the changes in ECs are referred to as *Bartonella* effector proteins (Beps). Work by Schulein *et al.* revealed that seven Beps (Beps A–G) are encoded downstream of the *virB/virD4* locus of *B. henselae* [44]. Each Bep contains at least one conserved, approximately 140 amino acid sequence at its C-terminus termed a *Bartonella* intracellular delivery (BID) domain, followed by a positively charged tail. If additional BID domains are present (e.g., in Beps E–G), they are apparently employed in effector functions rather than translocation [5]. Beps A–F also contain an N-terminal region, referred to as filamentation induced by cAMP (FIC) domain, whose function is unclear. Potential tyrosine phosphorylation domains are also present in Beps D–F [5], and phosphorylation has been demonstrated for BepD, following its translocation into the EC cytosol [44]. More recent work by Selbach *et al.* has shown that effector phosphorylation events constitute the foundation of an ‘interactome’, wherein a T4SS effector is ‘activated’ by a host cell tyrosine kinase [45]. In turn, the phosphorylated effector recruits and binds a variety of cellular proteins via their SH2 domains, to ultimately subvert various host cell activities that are mediated by cell signaling pathways. With regards to *Bartonella* effectors, phosphorylated BepE, BepF and BepD were found to interact with seven host cell proteins (Shp-1 and -2, Csk, Grb-2 and -7, Crk and Ras-GAP1) [45].

Characterizing the function(s) of the Beps is a fascinating topic currently under investigation by several labs. BepA has been shown to sufficiently inhibit EC apoptosis through its host cell membrane association and upregulation of cAMP levels in the cytosol [46]. Interestingly, BepA's BID domain can independently cause EC membrane localization and inhibition of apoptosis. More recently, BepG was shown to be required for invasome-mediated uptake of *B. henselae* into ECs by promoting cytoskeletal F-actin rearrangement, and the process is

induced at the expense of the more rapid, endocytic uptake and formation of BCVs [47]. The small GTPases Rac1 and Cdc42, but not RhoA, are required for subsequent invasome formation through their regulation of the changes that take place in the EC's actin cytoskeleton during internalization [47].

Pathological angiogenesis

The most notorious and unique manifestation of infection by the three major *Bartonella* species is the ability to induce pathological angiogenesis with concomitant production of pseudoneoplastic lesions in the human vasculature (i.e., verruga peruana, BA and bacillary peliosis). Although these lesions have been reported in immunocompetent patients [48], they are much more common in immunocompromised individuals infected with *B. henselae* and *B. quintana*. Therefore, immune status is a key risk factor [3,4]. By contrast, verruga peruana is a common manifestation of Oroya fever, regardless of immune status [49]. The mechanism whereby angiogenesis arises varies with *Bartonella* species and is multifactorial in nature (Figure 3).

B. bacilliformis

Early work with *B. bacilliformis* implicated a soluble, bacterial protein of over 12 kDa that was specifically mitogenic for cultured human umbilical vein ECs (HUVECs). HUVECs treated with the factor showed proliferation rates that were approximately threefold greater than controls. This study also demonstrated that the factor was able to elicit neovascularization into a surgically implanted sponge in rats [50].

A subsequent study by this group showed that live *B. bacilliformis* was able to stimulate proliferation of HUVECs in cocultures [51]. Later research revealed that GroEL, a chaperonin, is involved in *B. bacilliformis*' mitogenicity for cultured HUVECs. Interestingly, *B. bacilliformis* GroEL is actively secreted by the bacterium, and does not affect apoptosis of the host cell through inhibition of the caspase 3 pathway [52], as observed in *B. henselae* [53]. Whether GroEL is mitogenic or if it protects a mitogen(s) through its chaperonin activity is unclear; however, correlations between mitogenic cell fractions and GroEL's location within the bacterium (i.e., the soluble fraction is more mitogenic than the insoluble fraction), an increase in mitogenic activity following heat shock of the bacterium, and the ability of anti-GroEL and anti-GroES antibodies to decrease mitogenicity, suggest that GroEL is mitogenic for ECs. Using the same assay system, it is also clear that *B. bacilliformis*' mitogenic activity is significantly greater than *B. henselae*'s [52], underscoring disparities in the angiogenic mechanisms employed by different bartonellae. Interestingly, if GroEL is overexpressed by *B. bacilliformis* during intracellular infection of HUVECs it induces apoptosis, perhaps owing to the fact that it is an ortholog of mitochondrial heat-shock protein (Hsp)60; an inducer of programmed cell death [54].

B. henselae & B. quintana

Research with *B. henselae* and *B. quintana* has revealed a set of mechanisms involved in pathological angiogenesis that are distinct from those of *B. bacilliformis*, even though hemangiomatous lesions from all three organisms (i.e., verruga peruana and BA) are virtually indistinguishable (Figure 3).

The importance of a TAA in angiogenesis was first demonstrated in *B. henselae*, where activation of hypoxia-inducible factor (HIF)-1, a key proangiogenic regulator, was dependent on BadA expression [28]. Subsequent work showed that HIF-1 was activated in HUVECs and epithelial cells infected by BadA-expressing *B. henselae* [55]. More recent work has shown that a *B. henselae* strain expressing just the head domain of BadA can induce a proangiogenic

state in cocultured epithelial cells (HeLa 229), as gauged by HIF-1 activation and secretion of VEGF and IL-8, relative to uninfected controls [30]. In a similar study with *B. quintana*, TAA-deficient strains (*vomp*) were unable to induce a proangiogenic state (as measured by expression of VEGF in cocultured THP-1 and HeLa229 cells) [32].

Nuclear factor-κB activation during the inflammatory response in infected ECs was demonstrated by Fuhrman *et al.* [56]. Live *B. henselae*, or their purified omps, were able to cause EC activation as evidenced by NF-κB activation, NF-κB-dependent upregulation of E-selectin and ICAM-1 and, enhanced rolling and adhesion of polymorphonuclear neutrophils to the activated ECs. Proinflammatory conditions undoubtedly provide for increased chemotaxis and infiltration of potential effector cells (monocytes, macrophages and, possibly, polymorphonuclear neutrophils) to establish chronic inflammation and proangiogenic conditions.

Early work with *B. henselae* showed enhanced proliferation and migration of HUVECs in coculture and identified a trypsin-sensitive mitogen in the insoluble fraction of bacterial lysates [57]. However, subsequent work showed that the proliferative factor was actually secreted into the culture medium and was specific to ECs [58]. More recent work suggests that inhibition of apoptosis via BepA activity, not mitogenicity, is the means by which *B. henselae* increases the number of HUVECs in coculture [46]. Data also show that an antiapoptotic factor is released into the bacterial culture medium and, as previously demonstrated, is specific for ECs [53].

Additional studies have shown that *B. henselae* infection induces the expression of potentially angiogenic cytokines and growth factors *in vitro* that may act in a paracrine and/or autocrine fashion to stimulate the growth of ECs. Kempf *et al.* showed that TAA (BadA)-expressing *B. henselae* (referred to as ‘piliated’ at this time) induce the synthesis of VEGF by EA.hy 926 or HeLa cells, but not HUVECs, in cocultures [59]. HUVEC cultures supplemented with conditioned medium from EA.hy 926–*B. henselae* cocultures showed proliferation rates of 30–70-fold greater than controls. Kempf *et al*. also showed that EA.hy 926–*B. henselae* cocultures induced synthesis of IL-8, another angiogenic factor and chemotaxin [60]. Finally, this study demonstrated that *B. henselae* growth correlated with host cell growth rates, suggesting that paracrine stimulation might provide additional host cells for bacterial colonization. Resto-Ruiz *et al.* demonstrated that VEGF and IL-1β could be detected within 6–12 h in medium obtained from THP-1 macrophage cells cocultured with *B. henselae*, and that the conditioned medium was mitogenic for human microvascular ECs (HMEC-1s) [61]. Interestingly, HMEC-1s, but not THP-1 cells, were stimulated to produce IL-8 within 6 h of infection. McCord *et al.* demonstrated that *B. henselae* or purified low Mr omps can upregulate monocyte/macrophage chemoattractant protein-1 (MCP-1) production by cultured HMEC-1s [62]. The MCP-1 could conceivably attract monocyte–macrophage effector cells into the area of *Bartonella* infection and elicit a proangiogenic environment through their VEGF secretion. In a subsequent study, McCord *et al.* demonstrated that a variety of cell types (HUVECs, HMEC-1s, THP-1 monocytes and HepG2 hepatocytes) produced IL-8 in response to a *B. henselae* infection and that infected ECs upregulated expression of the IL-8 receptor, CXCR2 [63]. Infected ECs were also shown to form capillary tubes *in vitro* [63].

Finally, new research has shown that *B. henselae*'s T4SS effector, BepA (in addition to inhibiting apoptosis [46]) or just BepA's C-terminal portion containing a BID domain and charged carboxyl terminus, promotes capillary tube (‘sprout’) formation by HUVEC spheroids [64]. *B. quintana*'s ortholog (BepA2) was also shown to possess similar activity. By contrast, *B. henselae* BepG inhibits sprout formation (and also triggers extensive cytoskeletal rearrangement in ECs [46]). Interestingly, BepD also possesses stimulatory activity but at a lower level than BepA [64]. These data suggest that modern *Bartonella* species, with their

T4SS, may help regulate angiogenic activity through opposing activities of BepA/BepD and BepG effectors.

Taken as a whole, data suggest that pathological angiogenesis induced during a *Bartonella* infection:

- Is triggered by TAA-mediated activation of HIF-1 in host cells;
- Involves antagonistic T4SS effectors–BepA/BepD versus BepG in modern *Bartonella* to promote or inhibit sprout formation of ECs, respectively;
- Is fostered by BepA-mediated inhibition of EC apoptosis;
- Is enhanced by a proinflammatory microenvironment involving NF-κB activation in infected ECs;
- Involves paracrine stimulation with effector cell-secreted VEGF, IL1β and IL-8;
- Involves autocrine stimulation with IL-8 from infected ECs;
- Is augmented by soluble bacterial protein effectors (mitogens) secreted by *Bartonella* during infection (Figure 3).

Replication & persistence in the host

Heme acquisition

A hallmark characteristic of all *Bartonella* species is a requirement for heme, owing to their inability to synthesize protoporphyrin IX (PPIX) or heme (Fe^{2+}–PPIX). Common sources of heme include hemin (Fe^{3+}–PPIX), hemoglobin and host erythrocytes. However, unlike many pathogens, *Bartonella* cannot utilize heme-rich scavenger molecules of the host, such as lactoferrin or transferrin. Research has shown that acquisition of heme involves two main sets of virulence determinants including a paralagous gene family encoding Hbps (Pap31) and a heme uptake locus (Figure 4).

The Hbps are encoded by three (*B. bacilliformis*) or five (*B. quintana* and *B. henselae*) paralagous genes that are homologs to the group 3 *omp* gene family of *Brucella* (Table 1) [65]. The *hbp* genes of *B. quintana* are differentially expressed in response to ambient heme concentration and temperature, and they are positively regulated by the α-proteobacterial iron response regulator (Irr). Interestingly, *Bartonella* utilizes a unique promoter regulatory element for Irr binding, termed an ‘H-box’ [66]. Two subsets of *hbp* genes have been identified, including those induced under conditions resembling a human (e.g., *hbpA, hbpD* and *hbpE* at 37°C and low heme), and *hbpC*, which is induced under conditions simulating the louse vector (25°C, high heme) [67]. Hbps are heme-binding omps [68], however, their role in heme uptake is unclear, despite a predicted β-barrel structure that resembles porins [69]. A previous report demonstrated complementation of *E. coli hemA* using cloned *B. henselae hbpA* [70]. However, this complementation study was done at a heme concentration that may have caused outer membrane leakiness and given a false-positive reaction [70]. Furthermore, heme translocation by Gram-negative bacteria is normally TonB-dependent, but the Hbps lack a consensus TonB box for TonB energization of heme transport. With these caveats, one potential role for the Hbps is to provide a surface reservoir of heme. This arrangement would resemble closely related rhizobia, which bind plant-derived leghemoglobin to their surface in order to establish an antioxidant barrier through heme's intrinsic peroxidase activity. It would also provide a reservoir for the essential growth factor.

A more likely candidate for heme acquisition and transport is the heme utilization locus of *Bartonella*. This multiprotein heme-acquisition machine closely resembles those described in other Gram-negative bacteria and consists of a TonB-dependent heme receptor (heme

utilization locus [Hut]A), an ABC transport system (HutB, HutC and HmuV), a possible heme degradation/storage protein (HemS) and a TonB transducer for energizing HutA and other omps [71]. In *B. quintana*, the locus encoding this complex is repressed under high hemin concentrations and is negatively regulated by Irr. The *hemS–hutB–hutC–hmuV* genes are cotranscribed and located immediately downstream of *hutA*. Like the *hbp* genes, an 'H-box' for Irr regulation is present in the divergent promoter region that separates *hutA* and *tonB*, and in the promoter region upstream of the *hemS–hmuV* operon. Complementation analyses demonstrate that *B. quintana* HutA can serve as a heme receptor in *E. coli hemA* at low concentrations and that heme transport is TonB-dependent [71].

Coping with intracellular stress

The intracellular environment requires significant adaptations to cope with a long list of stressors, including reactive oxygen species, changes in pH, fluctuations in osmolarity and misfolded proteins. *Bartonella* would also be subject to temperature fluctuations when cycling between mammal hosts and arthropod vectors. As mentioned earlier, hemin bound to surface Hbps may provide an antioxidant barrier and nutritive reservoir for *Bartonella*. In addition, early work on the invasion-associated locus of *B. bacilliformis* revealed two genes immediately upstream of *ialB*, whose corresponding proteins have been shown to serve as antistress effectors in other pathogenic bacteria, including a nudix hydrolase (*ialA*) and a carboxy-terminal processing protease (CtpA).

Bartonella's nudix hydrolase (*ialA*) is a representative from a large family of related enzymes that hydrolyze (di)nucleoside polyphosphate alarmones (e.g., Ap4A, p4A) produced during nutritional, oxidative and temperature stress in cells (Figure 5). The products of hydrolysis include ATP and inorganic phosphate, which are subsequently recycled by the bacterium [72]. *ialA* orthologs have been implicated as virulence determinants of *B. bacilliformis* and *E. coli* during invasion of human erythrocytes and microvascular endothelial cells, respectively [19, 73]. Work with *Salmonella enterica* serovar Typhimurium has also shown that deletion mutants for *ygdP* and *apaH* (*ialA* homologs) display a significant increase in Ap4N levels and a concomitant decrease in invasiveness for cultured, human epithelial cells [74]. Although not well studied, research involving bacterial pathogens indicates that maintenance of NpnN levels by nudix hydrolases, such as *ialA*, is essential to an invasive phenotype [75].

Early work also identified a gene immediately upstream of *ialA* that encodes a tail-specific protease that possesses a conserved PDZ domain, called CtpA [76]. In a role that is similar to IalA, CtpA (and related COG0793 members) degrade aberrant or misfolded proteins that arise from stress or anomalous processing (Figure 5). Orthologs of CtpA have been shown to play a role in *Salmonella enterica* serovar Typhimurium's survival in macrophages [77], and possibly enhancing a mucoid phenotype in cystic fibrosis-associated strains of *Pseudomonas aeruginosa* by degrading aberrant forms of MucA, an alginate production regulator [78]. Although tail-specific proteases are typically located in the periplasm, CtpA may actually service both the cytosol and periplasm of *Bartonella* by translating two forms of CtpA using alternative start sites [76].

Another coping mechanism that warrants further exploration is the two-component regulatory system of *Bartonella*, and its role in regulating genes involved in virulence and countering stress imposed by the host and arthropod vector. Termed *Bartonella* two-component regulator/sensor (BatR/BatS) by its discoverer [BIRTLES R, UNPUBLISHED DATA], the BatR/BatS is an ortholog of *Brucella*'s BvrR/BvrS two-component regulatory system. In *Brucella*, BvrR/BvrS is involved in the invasion of host cells and maintenance of semi-permeability and homeostasis of the outer membrane [79]. *Brucella* mutants for either gene (*bvrR* or *bvrS*) are rapidly eliminated from infected mice and are unable to replicate within macrophages or epithelial cells [80]. BvrR's role in regulating the group 3 *omp* gene family [81] (homologs to

Bartonella's *hbp* gene family) suggested early on that a similar regulation might exist between BatR/BatS and the *hbp* genes. Unsurprisingly, recent work has shown that *B. henselae*'s BatR is an important transcriptional regulator during infection of ECs, where the regulon includes several virulence genes including the *hbp*s, T4SS and TAAs [DEHIO M *ET AL.*; UNPUBLISHED DATA]. Interestingly, BatR/BatS serves as a pH sensor that upregulates genes at neutral pH and represses them at alkaline pH; possibly evolved to respond to environmental cues encountered by *Bartonella* in the context of the mammalian circulatory system and arthropod vector, respectively.

Pacifying the immune system

Arguably the most difficult hurdles for a pathogen to overcome in order to establish a chronic, persistent infection is the host's innate and adaptive immune responses. Two general strategies are employed by pathogenic microorganisms in this regard; they can attempt to dismantle immune system effectors or their regulation using a variety of strategies (molecular mimicry, synthesis of superantigens to induce a ‘cytokine storm’, secretion of IgA protease and so on), or they can underwhelm the immune system by utilizing subinflammatory or antagonistic molecules. *Bartonella* employs the latter strategy, as exemplified by its low-potency lipopolysaccharide (LPS). The absence of classical manifestations of endotoxic shock during persistent infections of bartonellosis is evidence to this effect.

The LPS of *Bartonella henselae* has a deep-rough structure without an O-chain polysaccharide and contains an unusual penta-acylated lipid A with a long-chain fatty acid [82]. The absence of O-side chain could conceivably decrease complement fixation and provide a degree of serum resistance on *Bartonella*, but this possibility has not been explored. The unusual fatty acid composition renders *Bartonella*'s endotoxin at least 1000-fold less potent at Toll-like receptor (TLR)4 activation (as measured by IL-8 production), as compared with LPS from *Salmonella* [82]. Remarkably, *Bartonella*'s LPS possesses antagonistic properties for TLR4 and does not activate TLR2 [82,83]. These LPS attributes undoubtedly contribute to the establishment and maintenance of persistent infection, since the bacterium's major surface component is subinflammatory and antagonistic to the host's innate immune response. Interestingly, long-chain fatty acids are a conserved feature in the LPS of intracellular bacteria that establish long-term symbioses with their host, including *Legionella*, *Chlamydia* and closely related rhizobia.

Future perspective

Understanding the complete story of *Bartonella*'s molecular pathogenesis obviously requires more research. Specific areas that are ripe for investigation include: identification of virulence determinants used by the ancestral lineage, *B. bacilliformis* (which is comparatively much more virulent), versus those employed by more modern *Bartonella* species, such as *B. henselae* and *B. quintana* (which possess a T4SS), and how the T4SS has moderated virulence; continued work on characterizing differential mechanisms involved in pathological angiogenesis induced by ancestral versus modern *Bartonella* species; a more thorough analysis of the BatR/BatS two-component regulatory system and its virulence-associated regulon; elucidating, in detail, the functional relatedness between TAAs and other putative autotransporters from various *Bartonella* species; analysis of virulence gene expression and function in arthropod vectors (fleas, lice and sandflies); characterization of the remaining Bep T4SS effectors and analysis of their roles in EC subversion; and clarification of a handful of opaque issues including: deformin's biochemical nature, IalB's mechanism of enhancing erythrocyte invasion and deciphering HemS's role in cytosolic heme degradation or storage. Challenges to progress include a lack of alternative and inexpensive animal models of infection for human-specific *Bartonella* species (e.g., *B. quintana* and *B. bacilliformis*). Although the macaque is a useful model for these bartonellae, it is both expensive and labor-intensive to use. A second hurdle

is to counter the current decline in interest to support basic research on *Bartonella*. The funding problem has undoubtedly been compounded by the current global economic situation, and the fact that these so-called 'emerging' agents have been recognized for almost two decades. Considerable work remains to be done on these fascinating pathogens.

Executive summary

History

- A total of 24 *Bartonella* species have been described to date. Ten are pathogenic for humans and three species (*Bartonella bacilliformis*, *Bartonella quintana* and *Bartonella henselae*) are the most frequent causes of bartonellosis. These pathogens can cause intraerythrocytic bacteremia, pathological angiogenesis and multisystemic involvement.
- *B. bacilliformis* is the ancestral-type species; infections have been chronicled since pre-Columbian times by people in endemic regions of South America.
- *B. quintana* is the agent of trench fever and is re-emerging in homeless, inner-city populations where it causes 'urban trench fever'.
- *B. henselae* is the agent of cat-scratch disease and is emerging as a secondary pathogen of AIDS patients.

Transmission & colonization of humans

- *Bartonella* are transmitted by hematophagous arthropods (human body louse, cat fleas, phlebotamine sandflies and ticks) or animal trauma (scatches and bites).
- Humans are the sole reservoir for *B. quintana* and *B. baciliformis; B. henselae* infects a variety of mammals (e.g., cats, dogs and humans).

Erythrocyte parasitism

- All *Bartonella* species parasitize erythrocytes (hemotrophy).
- *B. bacilliformis* infection can cause an acute drop in hematocrit, whereas *B. quintana* and *B. henselae* cause chronic intraerythrocytic bacteremia without anemia.
- *B. bacilliformis* and, possibly *B. henselae*, secrete a hydrophobic factor (deformin), which pits and invaginates erythrocyte cell membranes.
- Based on work with *B. tribocorum*, Trw orthologs are likely to be used as erythrocyte adhesins by *B. quintana* and *B. henselae*.
- Flagella-mediated motility is used by *B. bacilliformis* to parasitize erythrocytes.
- IalB somehow enhances erythrocyte invasion and is maximally expressed under arthropod-like conditions.
- Hemolytic factors may enhance *Bartonella* hemotrophy, including a contact-dependent hemolysin and an autotransporter cohemolysin.

Endothelial cell parasitism

- *Bartonella* possess YadA-like outer membrane proteins (outer membrane proteins; afimbrial adhesins), designated trimeric autotransporter adhesins (TAAs) based on their structure and function in other bacterial pathogens.

- *B. henselae's* TAA (BadA) is an adhesin for endothelial cells (ECs) via β1-integrins and binds various extracellular matrix proteins. BadA also inhibits phagocytosis by J774 macrophages and causes autoaggregation.
- *B. quintana's* TAAs (variably-expressed outer membrane proteins) bind to collagen IV, cause autoaggregation and are essential in establishing chronic bacteremia in macaques.
- EC internalization of *B. bacilliformis*, and other *Bartonella*, involves the small GTPases, Cdc42, Rho and Rac.
- *B. henselae* internalization involves an invasome or endocytic uptake.
- *Bartonella* abrogate or inhibit lysosome fusion with the phagosome, creating a special vacuole, termed a *Bartonella*-containing vacuole.

Subverting the host cell

- *B. quintana* and *B. henselae*, but not *B. bacilliformis*, possess a type IV secretion system (T4SS) for delivery of Bep effectors.
- Delivery of Beps via the VirB/VirD4 T4SS is responsible for EC cytoskeletal changes, activation of NF-κB and inhibition of apoptosis.
- BepA is involved in EC membrane localization and inhibition of apoptosis. BepG enhances invasome formation in ECs at the expense of the endocytic pathway.

Bartonella-induced angiogenesis

- *B. bacilliformis* synthesizes a soluble, proteinaceous mitogen for human umbilical vein ECs (HUVECs) that is angiogenic *in vivo*.
- Extracellular *B. bacilliformis* GroEL is involved in mitogenicity for HUVECs, whereas excess, intracellular GroEL can trigger apoptosis.
- *B. henselae* infection results in activation of HIF-1, which in turn, induces proangiogenic mediators, such as VEGF and IL-8. *B. henselae* activation of HIF-1 requires its TAA, BadA.
- *B. henselae* inhibits apoptosis of HUVECs via T4SS-translocated BepA.
- *B. henselae* upregulates expression of VEGF in EA.hy 926, HeLa and THP-1 cells and IL-1β in cocultured THP-1 cells. VEGF and IL-1β probably act on ECs in a paracrine fashion.
- *B. henselae* induces IL-8 in THP-1, hepatocyte, EA.hy 926 and HMEC-1 cells. IL-8 probably acts on ECs in an autocrine and paracrine loops.
- *B. henselae* upregulates MCP-1 in HMEC-1 cells. MCP-1 probably recruits monocyte–macrophage effector cells to the site of infection.
- *B. henselae* BepA and BepD stimulate, whereas BepG inhibits, sprout formation by HUVEC spheroids.

Bacterial replication & persistence

- *Bartonella* have an absolute growth requirement for host-derived heme.
- Hbps bind heme to the bacterial surface, where it may provide an antioxidant barrier and reservoir for the nutrient.
- *Bartonella* utilize a heme-uptake complex that resembles those of other Gram-negative pathogens.

- (Di)nucleoside polyphosphate alarmones and misfolded proteins arising in the intracellular niche are degraded by IalA and CtpA, respectively.
- BatR/BatS, a two-component system, regulates several virulence genes expressed during EC parasitism.
- *Bartonella* lipopolysaccharide is subinflammatory; it is an antagonist for Toll-like receptor 4 and does not activate Toll-like receptor 2.

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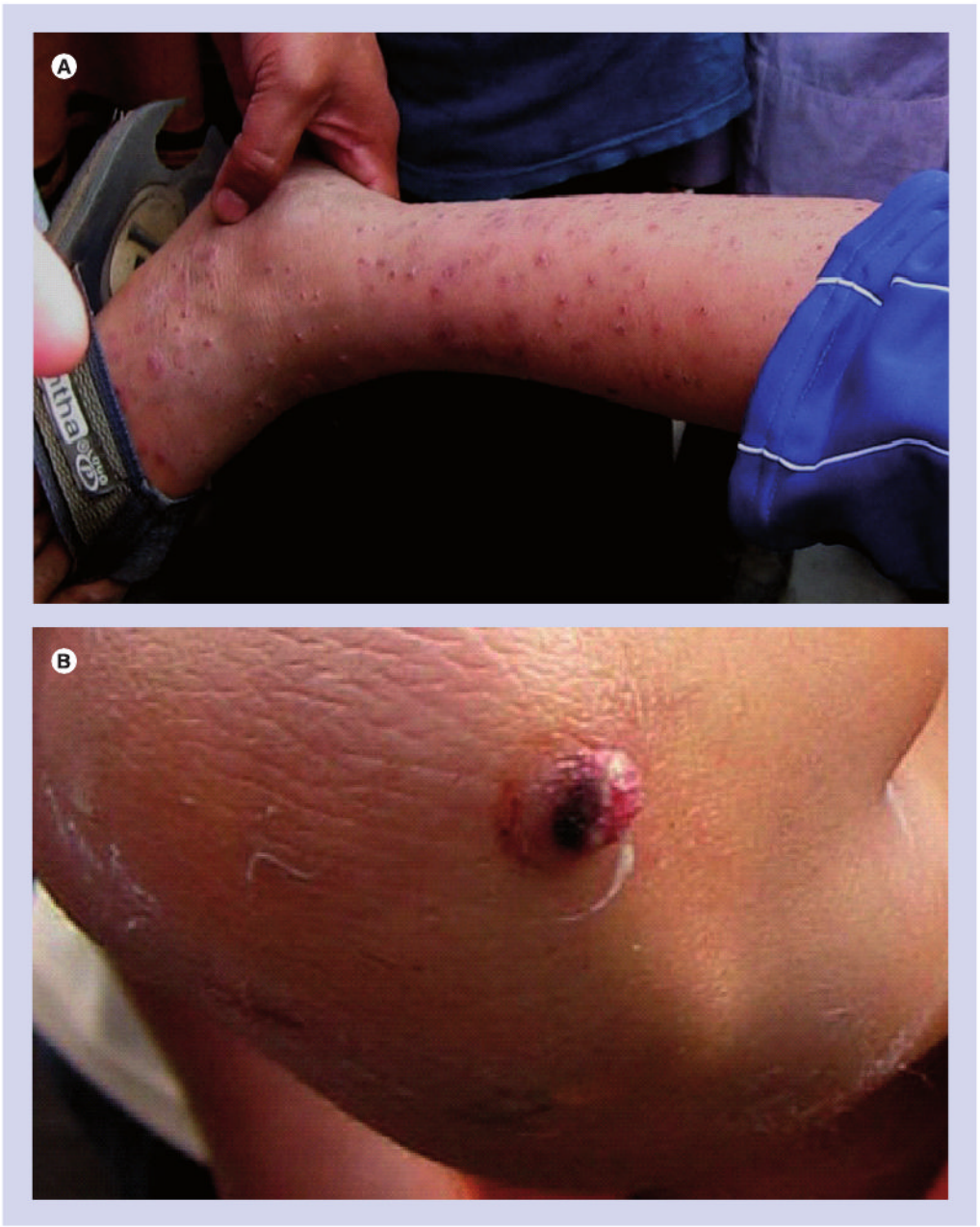

Figure 1. Angiomatous lesions caused *by Bartonella bacilliformis*
(A) Numerous verruga peruana and **(B)** a close-up of a lesion on a child living in a mountain village near Casma, Peru, following an outbreak of Oroya fever in 2008.
Courtesy of David Pascual.

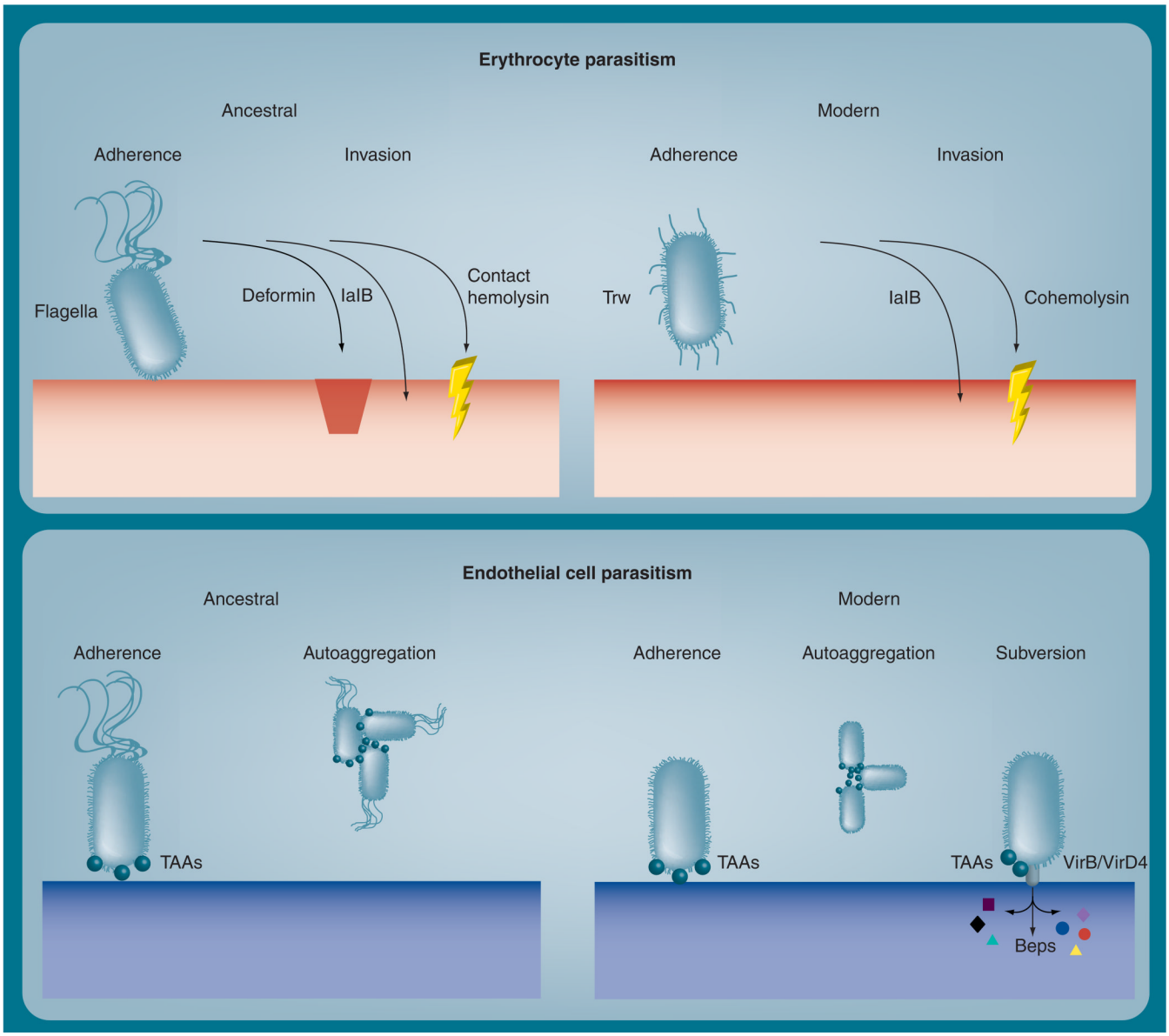

Figure 2. Virulence factors utilized by ancestral (*Bartonella bacilliformis*) and modern *Bartonella* species (*Bartonella henselae* and *Bartonella quintana*) to parasitize human erythrocytes and endothelial cells

A summary of the depicted determinants and their occurrence in pathogenic *Bartonella* are provided in Table 1.

Bep: *Bartonella* effector protein; TAA: Trimeric autotransporter adhesin.

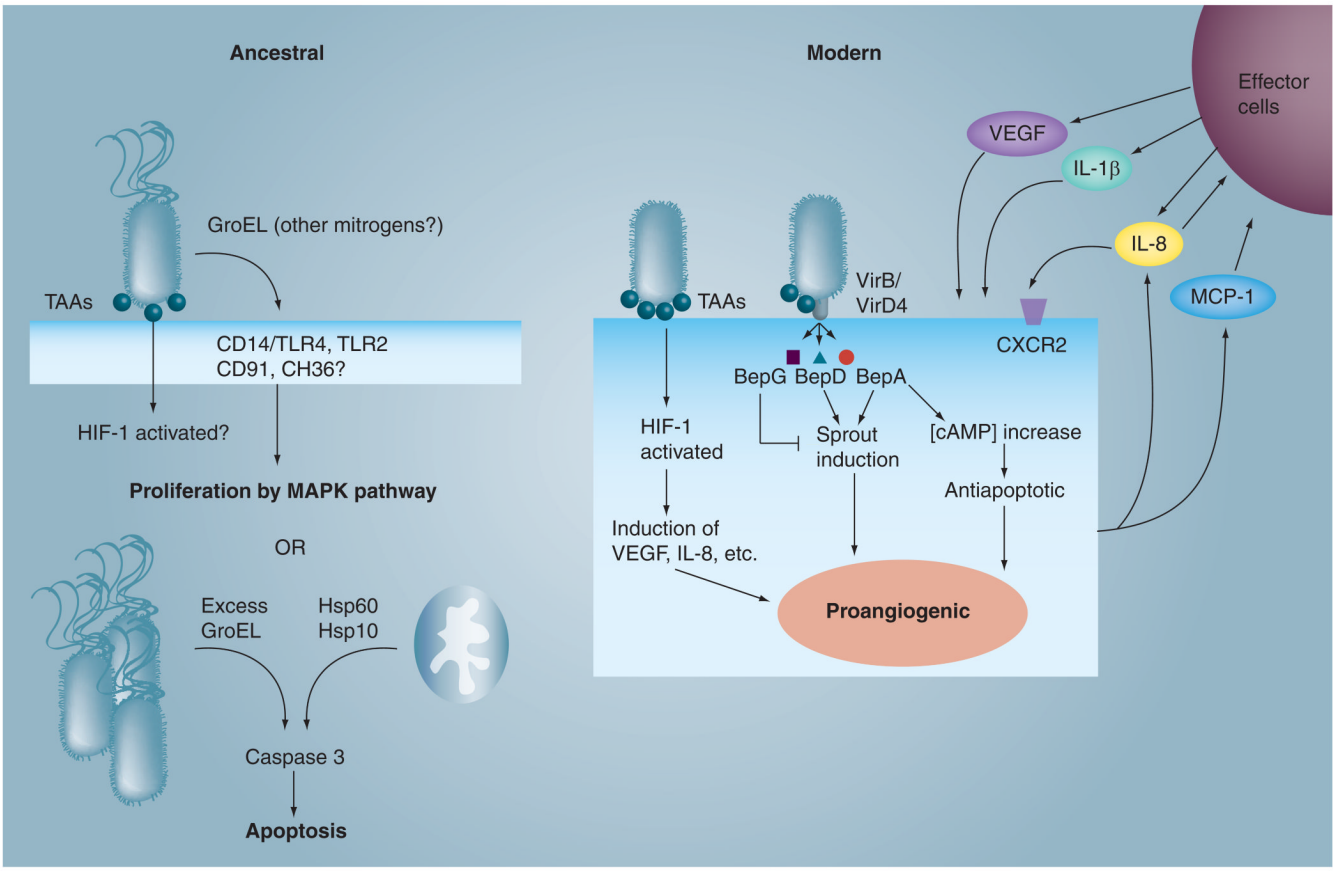

Figure 3. Potential effectors of ancestral (*Bartonella bacilliformis*) and modern *Bartonella* species (*Bartonella henselae* and *Bartonella quintana*) that trigger pathological angiogenesis in endothelial cells

TAAs are likely required by both ancestral and modern *Bartonella*, although little is known about the *B. bacilliformis* orthologs. Secreted, extracellular GroEL may serve as a *B. bacilliformis* mitogen [52], whereas excess GroEL produced by intracellular *B. bacilliformis* in human umbilical vein endothelial cells (ECs) induces apoptosis, perhaps because it is an ortholog of Hsp60 [54]. Modern *Bartonella* evoke proangiogenic conditions in ECs by activating HIF-1 through TAA (e.g., BadA) interactions with the host cell membrane [28,55]. In turn, activated HIF-1 induces the expression of several angiogenic mediators, including IL-8 and VEGF [54]. The VirB/VirD4 type IV secretion system delivers BepA to the cytosol, where it triggers an increase in cytosolic cAMP, which inhibits apoptosis [46]. In addition, both BepA and BepD induce sprout formation in ECs, whereas BepG is antagonistic to this process [64]. The entire infection is accompanied by activation of NF-κB, which contributes to a proinflammatory state [56]. MCP-1 and IL-8 from ECs are thought to serve as chemotaxins for recruitment of various effector cells – an additional source of VEGF, IL1β and IL-8 (top right hand corner).

Bep: Bartonella effector protein; CD: Cluster of differentiation; CXCR: CXC motif chemokine receptor; HIF: Hypoxia-inducible factor; Hsp: Heat-shock protein; GroEL: Molecular chaperone; MCP: Monocyte/macrophage chemoattractant protein; TAA: Trimeric autotransporter adhesin; TLR: Toll-like receptor.

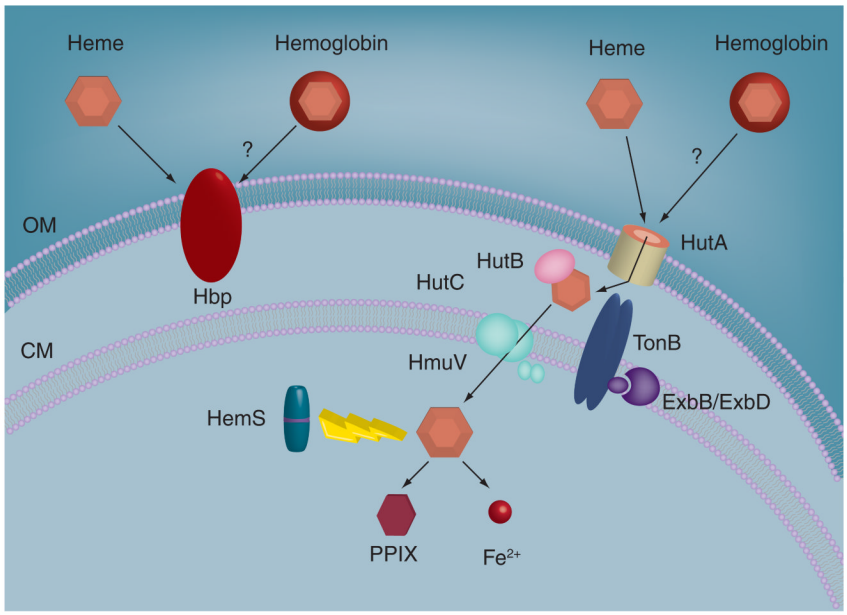

Figure 4. Heme acquisition system of *Bartonella*

A paralagous family of Hbps serves to bind heme to the surface, where it likely serves as an antioxidant barrier and nutrient reservoir. A heme receptor (HutA) is energized by TonB to transport heme to the periplasm where it is subsequently bound and shuttled by HutB to an ABC transporter/permease (HutC/HmuV). Transported heme is utilized for biosynthesis of heme-containing proteins or is stored or degraded by cytosolic HemS into ferrous iron and PPIX.

CM: Cytosolic membrane; Exb: Participant in TonB-mediated energy transduction for outer membrane proteins; Hbp: Heme-binding protein; HemS: Heme degradation/storage protein; Hmu: Heme import protein; Hut: Heme utilization locus; OM: Outer membrane; PPIX: protoporphyrin IX; Ton: Energy transducer for outer membrane proteins.

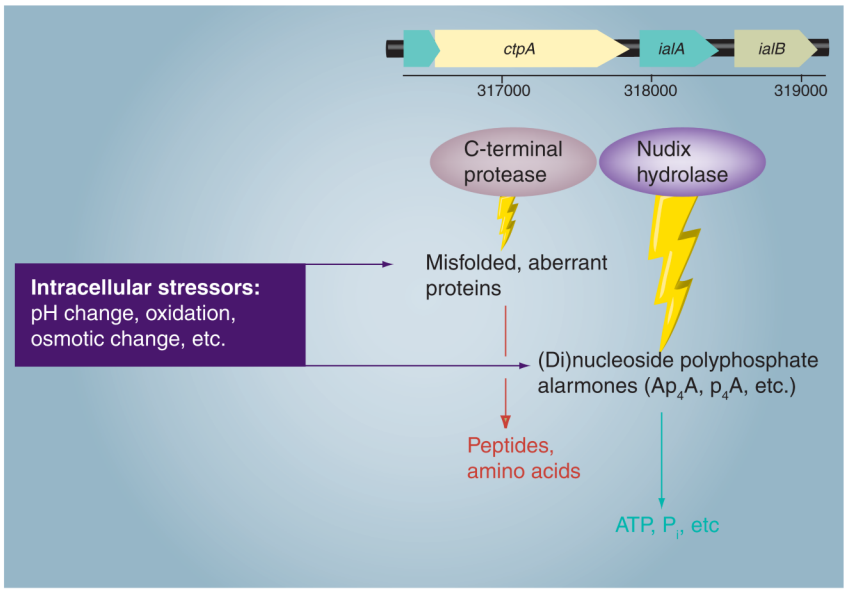

Figure 5. ***Bartonella*** **enzymes used to degrade misfolded or aberrant proteins and (di)nucleoside polyphosphate alarmones generated during parasitism of host cells, including a C-terminal protease (CtpA) and Nudix hydrolase (IalA)**

Genes for these enzymes are located immediately upstream of *ialB*, a gene involved in erythrocyte parasitism (a linkage map from *Bartonella bacilliformis* is shown).

Table 1

Alphabetical listing of proven or likely virulence determinants of Bartonella, based on current literature, phenotypes (contact hemolysin, deformin and LPS) and GenBank listings (all others)

Virulence determinant	*Bartonella bacilliformis*	*Bartonella quintana*	*Bartonella henselae*
BatR/BatS	Present	Present	Present
Cohemolysin	?	Present	Present
Contact hemolysin	Present	Absent	Absent
C-terminal protease	Present	Present	Present
Deformin[*]	Present	?	?
Flagella-mediated motility	Present	Absent	Absent
Hbp family[‡]	Present[§]	Present[§]	Present[§]
Heme uptake system[¶]	Present[#]	Present	Present
IalA/IalB[**]	Present	Present	Present
LPS with low endotoxicity	Present	Present	Present
TAAs[*‡]	Present	Present	Present
Trw pilin system	Absent	Present	Present
VirB/VirD4 and Beps[*§]	Absent	Present	Present

[*] Deformin (deformation factor) has been reported once for *Bartonella henselae* [13].

[‡] Heme-binding proteins. Also referred to as phage-associated protein of 31 kDa (Pap31) of *B. henselae*.

[§] Three Hbps in *Bartonella bacilliformis*, five in both *B. henselae* and *Bartonella quintana*.

[¶] Heme receptor, periplasmic heme-binding protein, ABC transporter/permease and TonB.

[#] A TonB ortholog is not encoded in this genome; possibly uses TolC.

[**] Invasion-associated locus A and B for a nudix hydrolase and erythrocyte invasion protein, respectively.

[*‡] Trimeric autotransporter adhesins [26]; an orthologous group of YadA-like outer membrane proteins/afimbrial adhesins. Designated as variably-expressed outer membrane proteins in B. quintana, Bartonella repetitive protein A in *Bartonella vinsonii*, and *Bartonella* adhesin A in *B. henselae*.

[*§] Type IV secretion system and translocated Beps.

BatR: *Bartonella* regulator; BatS: *Bartonella sensor;* Bep: *Bartonella* effector protein; Hbp: Heme-binding protein; LPS: Lipopolysaccharide; TAA: Trimeric autotransporter adhesin.