J. Med. Microbiol. — Vol. 51 (2002), 915–923

ISSN 0022-2615

REVIEW ARTICLE

Bartonella: new explanations for old diseases

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Introduction

Until recently, there were only two recognised human diseases caused by *Bartonella* spp.: trench fever due to *B. quintana* and Carrion's disease due to *B. bacilliformis*. Since then, *Bartonella* spp. have been recognised as causative agents of further human diseases, including bacillary angiomatosis, cat-scratch disease, chronic bacteraemia, chronic lymphadenopathy, meningoencephalitis, stellar retinitis, myelitis, granulomatous hepatitis, endocarditis, osteomyelitis and peliosis hepatitis [1] (Table 1). In parallel, the genus *Bartonella*, which until 1993 contained only one species (*B. bacilliformis*), was broadly extended by reclassifying within it the genera *Rochalimea* [2] and *Grahamella* [3], and by the description of new *Bartonella* species (Fig. 1). Based on phylogenetic analysis of the 16S rRNA sequences, the relatedness of *Bartonella* spp. to other alpha-2 *Proteobacteria* including *Brucella* spp., *Afipia* spp., *Agrobacterium tumefaciens*, *Bradyrhizobium* spp. and *Bosea* spp. has been demonstrated [2, 3]. Active research on the pathogenesis of *Bartonella* infections has been triggered by the increased number of species of *Bartonella*, the re-emergence of older disease due to *Bartonella*, such as the modern form of trench fever that affects alcoholics and homeless people [4], the recognition of the role of *Bartonella* in AIDS-related diseases and the description of new clinical entities due to *Bartonella* spp. As a facultative intracellular bacterium, *Bartonella* interacts closely with its host cells. The study of these interactions gave an insight into some of the underlying virulence factors and pathogenic mechanisms, which may be common or specific to the host cell or to the *Bartonella* species studied, or both. Interactions of *Bartonella* spp. with (i) red blood cells and (ii) endothelial cells have been studied for several years, but (iii) bone marrow progenitor cells may also play a central role as a sanctuary site in the pathogenesis of *Bartonella* (Fig. 2).

Received 24 May 2002; accepted 4 June 2002.
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The red blood cell

Infection of the red blood cell: a persistence and dissemination strategy

Bartonella infections are vector-borne diseases characterised by a natural cycle, vectors and reservoir hosts [5]. Human pathogenicity is either related to natural infection (*B. quintana* and *B. bacilliformis*) or to incidental infections (for other *Bartonella* spp.), acquired through contact with naturally infected mammals. Many *Bartonella* spp. have been shown to multiply and persist in red blood cells, sharing common persistence and dissemination strategies. Thus, *B. henselae* was found to infect cat red blood cells [6–8] and *B. tribochorum* those of rats [9], whereas *B. quintana* [10] and *B. bacilliformis* [11] were shown to invade human red blood cells. Bacteraemias were described even in healthy mammals, thus being one established exception to one of Koch's basic statements: 'Bacteria do not occur in the blood or tissues of healthy animals or humans' [12]. It was shown in the red blood cells of rats that the bacterial replication of *B. tribocorum* was regulated and stopped at a maximum of eight bacteria/cell [9]. The mechanism of regulation is unknown, but it prevents haemolysis and allows the persistence of *Bartonella* spp. within red blood cells. Disregulation of this mechanism, with uninterrupted multiplication of *B. bacilliformis* within red blood cells, might explain the rare acute haemolytic form of Carrion's disease (Oroya fever). However, as treatment of *Bartonella* with proteinase K has been shown to reduce the haemolytic activity of *B. bacilliformis* by 25%, a bacterial protein may also be involved in Oroya fever-associated haemolysis [13]. The ability of *Bartonella* to persist in erythrocytes for long periods may have been driven by evolutionary constraints, as it increases its transmissibility by blood-sucking arthropods. This persistence, which explains the prolonged bacteraemia in symptomatic and asymptomatic subjects, and the occurrence of disseminated

Table 1. *Bartonella* infections in man and related clinical entities

Disorder	Clinical presentation	Species involved
Carrion's disease		
Oroya fever	Acute febrile haemolytic anaemia or mild fever with body pain, nausea and headache	*B. bacilliformis*
verruaga peruana	Exophytic or miliary skin eruption	*B. bacilliformis*
Trench fever	Relapsing fever, headache and body pain	*B. quintana*
Bacillary angiomatosis	Red and papular cutaneous lesions (with or without lymphadenopathy, osteolysis, fever, weight loss)	*B. quintana*, *B. henselae*
Bacillary peliosis	Abdominal pain, fever, hepatosplenomegaly	*B. henselae*
Cat-scratch disease	Lymphadenopathy (with or without mild fever, body pain and headache)	*B. henselae*, *B. clarridgeiae*?
ocular involvement	Conjunctival ulceration, stellar retinitis, neuroretinitis	*B. henselae*, *B. grahamii*
Chronic bacteraemia	Fever, headache, leg pain and thrombocytopenia	*B. quintana*
Endocarditis	Fever, dyspnoea on exertion, bibasal lung rales, cardiac murmur, embolic phenomena and vegetations	*B. quintana*, *B. henselae*, *B. vinsonii*, *B. elizabethae*

disease in the homeless (chronic bacteraemia) or AIDS patients (bacillary angiomatosis), may also be favoured by the intra-erythrocytic localisation which partially protects *Bartonella* from the immune system.

Adherence to red blood cells

B. bacilliformis possess polar flagella (Fig. 3) that have been shown to mediate erythrocyte adhesion [14]. This flagella-associated adhesion was supported by the poor adherence of non-motile variants [11] and has been confirmed by the reduction of the erythrocyte-binding ability of *B. bacilliformis* by 40–50% with antiflagellin antibodies [15] and by the 75% reduction in binding ability of a flagellin-minus mutant [16]. Importantly, erythrocyte adhesion was not fully restored by complementation of the minus-mutant [16]. As the other *Bartonella* spp. (except *B. clarridgeiae*) do not possess polar flagella and as the *B. bacilliformis* flagellin-minus mutant retained some adhesive ability, other important mechanisms are involved in adherence to red blood cells. One of these may be the bundle-forming pili expressed at the surface of low-passaged *B. henselae* and *B. bacilliformis* cells, which have been implicated in the auto-agglutination propensity of *Bartonella* spp. [17]. Proteins exposed at the bacterial surface may also play a role in adhesion to erythrocytes, although no proteins able to interact with red blood cells have been identified yet. As adherence of *B. bacilliformis* to red blood cells is inhibited when the bacteria are pre-treated with N-ethyl maleimide, but is not inhibited when the erythrocytes are pre-treated with the same reagent [14], the red blood cell seems to play only a passive role in adherence. Membrane erythrocyte proteins, which bind *B. bacilliformis* passively, include spectrin and glycophorins A/B [18]. Enhanced binding of *Bartonella* to these proteins after treatment of erythrocytes with trypsin or neuraminidase has suggested a possible masking of binding site [18]. The fact that glycophorin A/B is the major receptor on human erythrocytes for *Plasmodium falciparum* merozoites [19] suggests that this protein is also utilised by *Bartonella* to bind to erythrocytes.

Invasion of red blood cells

It has been speculated that the binding of *Bartonella* to spectrin may be a first step necessary to alter the erythrocyte membrane for internalisation of *Bartonella* cells [18]. This hypothesis is supported by the role of spectrin in the maintenance of erythrocyte shape and membrane integrity and deformability [18] and by the fact that proteases from *P. falciparum* were shown to cleave erythrocyte cytoskeletal components and spectrin [20]. A bacterial protein named deformin also appeared to be involved, at least for *B. bacilliformis,* in the formation of pits and trenches in the red cell membranes [11, 21], that may favour both colonisation and entry into the cell [17]. This molecule, initially thought to be a protein, seems to be a small hydrophobic molecule with affinity for albumin, although the effector mechanism remains to be elucidated [22]. The presence of a homologue of the deformin of *B. bacilliformis* that also led to the invagination of red blood cells, although less pronounced, has been identified in *B. henselae* culture supernate [23], suggesting that the deformin-mediated invasion mechanism might be shared between several *Bartonella* species.

At the molecular level, the genes of the **i**nvasion-**a**ssociated **l**ocus (*ial*) identified in *B. bacilliformis* have been shown to confer an invasive phenotype to minimally invasive *Escherichia coli* strains [24]. The *ial* contains two genes named *ialA* and *ialB*. Immediately upstream lies a gene encoding a carboxy-terminal protease, *ctpA,* and another gene encoding the filament A polypeptide (FilA) [17, 25]. Downstream lie two open reading frames that do not share homology with other species [17, 25]. These six genes are thought to constitute a pathogenicity gene cluster, as (i) both *ialA* and *ialB* genes are needed to confer the invasive phenotype to *E. coli*; (ii) the gene encoding for the

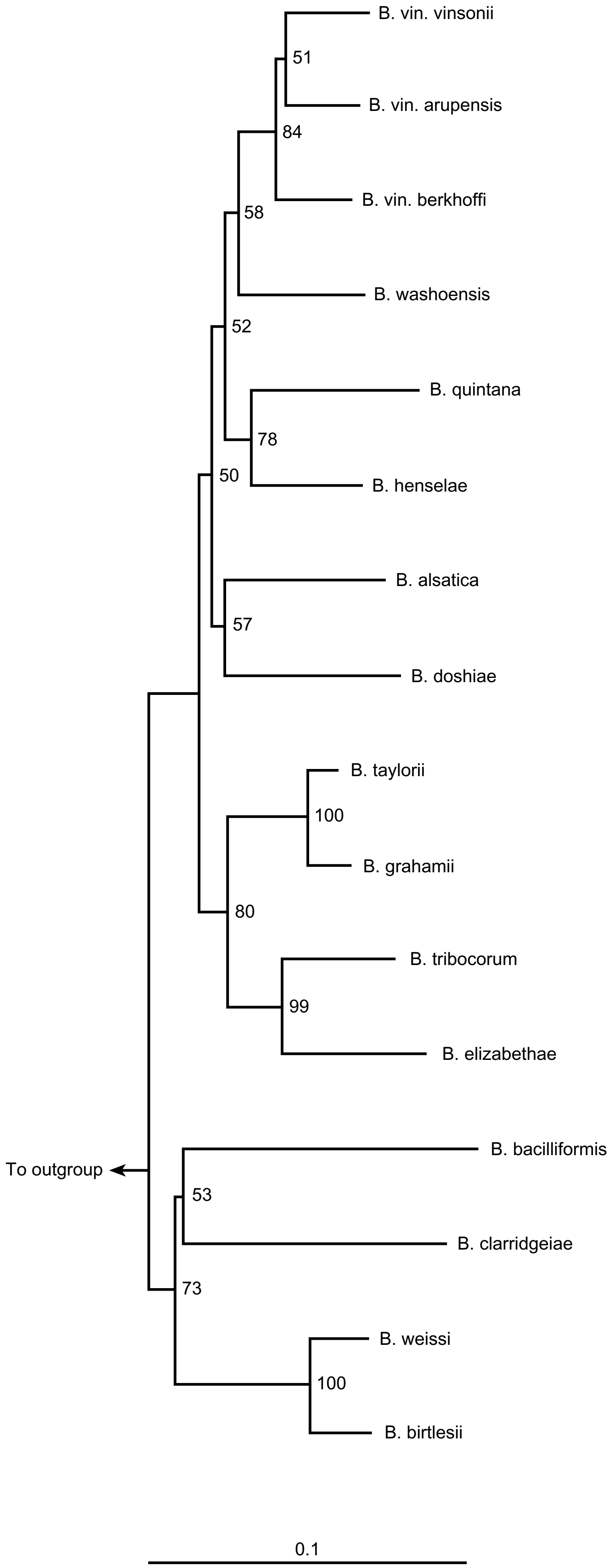

Fig. 1. Neighbour-joining tree resulting from comparison of sequences of groEL encoding genes of most *Bartonella* spp. identified to date. The values at each node represent the percentage of times each branch was found in 100 bootstrap replicates.

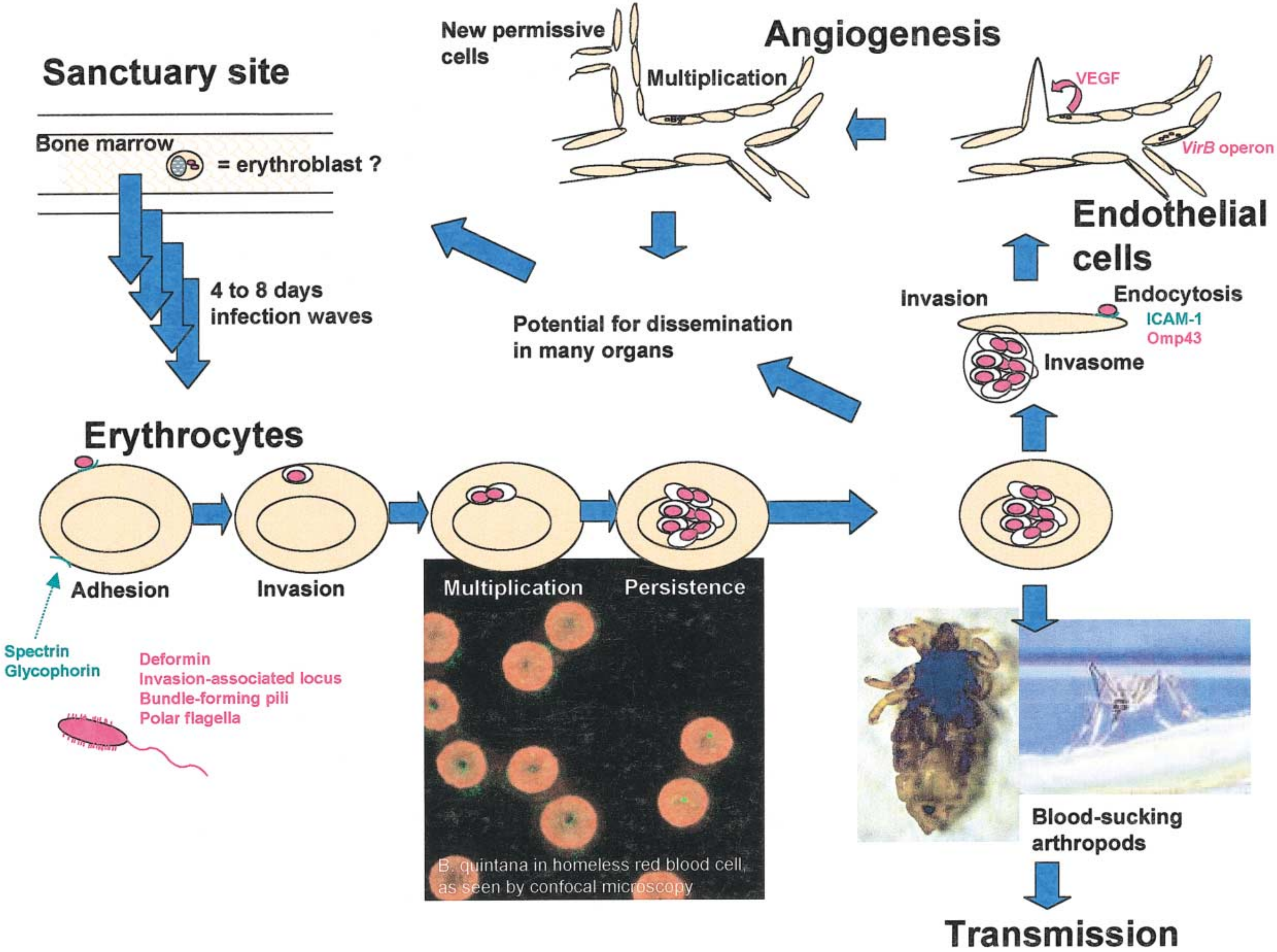

Fig. 2. Interactions of *Bartonella* spp. with red blood cells, endothelial cells and a putative sanctuary site located in the bone marrow. *Bartonella* and *Bartonella*-associated factors are represented in pink, host cell-associated factors are represented in green.

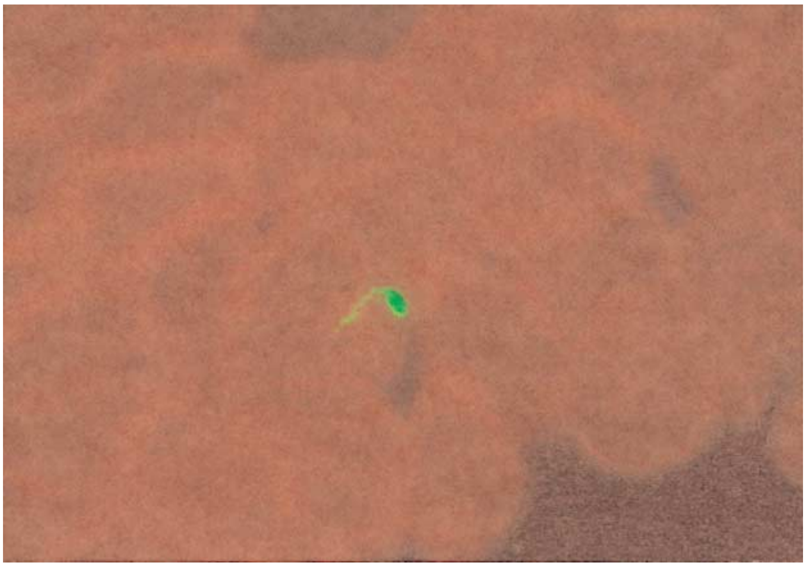

Fig. 3. *B. bacilliformis* and its polar flagella, within human erythrocytes, as seen by confocal microscopy. (Courtesy of J. M. Rolain, Marseille.)

adhesion and invasion locus (Ail) protein of *Yersinia enterocolitica*, which is implicated in host cell attachment and invasion, shares 60% amino acid similarity with that encoded by *ialB*; and (iii) FilA exhibits amino acid similarity with various filamentous proteins including the M1 of *Streptococcus pyogenes,* involved in adhesion and invasion [17]. Furthermore, *CtpA* might also be involved in virulence, as a C-terminal protease of *Salmonella typhimurium* enhances intramacrophage survival [26]. More importantly, several genes of this cluster have been sequenced in other *Bartonella* spp.*,* and also in other facultatively intracellular bacteria, suggesting that some of the adhesion, invasion and persistence mechanisms used by *Bartonella* may be common to these species. Thus, the amino acid sequence of *B. bacilliformis* ialA shared 73% homology with that of *B. clarridgeiae* and 49% with that of *Brucella melitensis,* another member of the Rhizobium group, while the amino acid sequence of *B. bacilliformis* CtpA shared 81% homology with that of *B. quintana* and 71% with that of *Br. melitensis* (homology analysis performed with ClustalW) [27]. Whether *ialA* and *ialB* may explain the peculiar tropism of *Bartonella* for red blood cells remains to be defined. The role played by *CtpA*, *FilA* and both additional orphan open-reading frames remains also to be clarified. A role in quorum sensing, i.e., regulation of the intra-erythrocytic growth of *Bartonella* may be possible. In conclusion, it appears that specific (polar flagella) and common (deformin, invasion-associated locus) mechanisms have been developed by the different *Bartonella* spp. to adhere to and invade the red blood cells.

Angiogenesis

Apart from their tropism for red blood cells, a second typical pathogenic feature of *Bartonella* spp. is their ability to trigger angiogenesis. Such pathological angiogenesis is observed in bacillary angiomatosis and peliosis [28–31].

An unknown protein

The first experimental evidence that *Bartonella*-related angiogenesis may be due to a protein was afforded by Garcia *et al*. in 1990 [32]. They demonstrated that *B. bacilliformis* extracts possess an activity that stimulates endothelial cell proliferation up to three times that of a control [32]. The factor, which was found to be specific for endothelial cells and was larger than 12–14 kDa (not dialysed), was thought to be a protein because it was heat sensitive and precipitated with ammonium sulphate 45% [32]. *B. bacilliformis* extracts were also reported to stimulate the production of tissue plasminogen [32]. Live bacteria were later shown to increase both parameters (angiogenesis and tissue plasminogen production) in a fashion similar to the homogenates of *B. bacilliformis* [33]. In 1994, Conley demonstrated in a similar in-vitro model that *B. henselae* induces an angiogenic factor, whose susceptibility to trypsin also suggests that the factor may be a protein. More recently, it has been shown that this factor may be secreted by *B. henselae* [34], indicating that *B. henselae* may induce endothelial cell proliferation independently of bacterial invasion. The dramatic effect of erythromycin on the cutaneous lesions of bacillary angiomatosis [35, 36] may be due to inhibition of the production of the proteic angiogenic factor(s), as erythromycin is known to inhibit protein production at the ribosomal level. An angiogenesis-based effect would better explain the rapidity of its effect and its lack of a sustained response than a direct microbicidal one.

Vascular endothelial growth factor (VEGF)

VEGF, angiopoietins and ephrins were proved to be critical and specific for blood vessel formation [37]. Recently, Kempf *et al*. demonstrated that *B. henselae* induce EA.hy 926 cells (permanent endothelial cell line expressing factor VIII) to produce VEGF, which in turn was able to stimulate the proliferation of endothelial cells and the growth of *B. henselae* [38]. In the same study, Kempf *et al*. showed that the administration of VEGF-neutralising antibodies reduced endothelial proliferation by 50% [38], suggesting that other factors are involved, such as angiopoietins, ephrins or the yet unknown proteic factor discussed above.

Importantly, VEGF not only induces endothelial proliferation but also cell migration through several pathways that include the activation of a small GTP-ase RhoA, which is associated with phosphorylation of myosin light chain [39]. This may easily explain why *Bartonella*-associated angiogenesis is also characterised by altered spatial organisation within the monolayer and changes in cell morphology due to cytoskeleton reorganisation [40]. However, the impaired migratory ability of *Bartonella*-infected endothelial cells [41] was more surprising. This impaired mobility might result from the formation of thick robust stress fibres, probably *via* the activation of RhoA, as suggested by an inactivation assay [41]. Thus, the cells that effectively participate in angiogenesis are probably uninfected endothelial cells that respond to VEGF produced by the infected ones. *B. henselae* not only promotes the formation of new permissive cells but also protects the infected cells from apoptosis. Indeed, *Bartonella* suppress both early and late events in apoptosis, namely caspase activation and DNA fragmentation [42]. This anti-apoptotic effect, found to be specific for endothelial cells [42], may be mediated at least partially by VEGF, which is known to protect the cells from apoptosis [43].

Peliosis hepatitis

Peliosis hepatitis, which corresponds histologically to multiple blood-filled cystic spaces often communicating with the hepatic sinusoids, was initially thought to be induced by viruses [44]. Similar lesions were later observed in mice or rats exposed to various drugs or toxins, including phalloidine and oxazepam [45, 46] and reported in patients with advanced cancer or receiving anabolic steroids. The *Bartonella*-associated peliosis hepatitis, associated with HIV infection, differs from that of classical peliosis hepatitis by the additional presence of clumps of bacteria [47]. Recently, while studying the ability of VEGF to block tumour regression, Wong *et al*. observed that mice implanted with VEGF-expressing tumours sustained high morbidity and mortality that were out of proportion to the tumour burden. High serum levels of VEGF were associated with a lethal hepatic syndrome characterised by massive sinusoidal dilatation and endothelial cell proliferation and apoptosis [48]. A striking reversal of VEGF-induced liver pathology was achieved by surgical excision of VEGF-secreting tumours or by systemic administration of a potent VEGF antagonist [48]. As this VEGF-induced syndrome resembles cancer and *Bartonella*-induced peliosis hepatitis, the bacillary peliosis hepatitis may be directly due to the VEGF produced by the infected endothelial cells and by other infected cells that are known to secrete VEGF, such as erythroblasts [49].

Placenta growth factor

The placenta growth factor (PIGF) is analogous to VEGF and is secreted by the placenta and erythroblasts [37, 49]. Its effect on *Bartonella* multiplication remains to be defined, as does the role played by *Bartonella* in its production. However, the presence of vascular lesions in the maternal placenta of mice infected experimentally with *B. birtlesii* [50], and the role played by VEGF in *Bartonella* pathogenesis [38], suggest that PIGF might be involved in the genesis of the reproductive disorders observed by Boulouis *et al*. in infected mice.

Endothelial cells

The *Bartonella*–endothelial cell interaction is not restricted to angiogenesis stimulation. Thus, (i) invasion of endothelial cells was described for *B. quintana* [51], *B. henselae* [52] and *B. bacilliformis* [53], and (ii) a pro-inflammatory activation of endothelial cells was postulated, which is thought to be a result of receptor-ligand interactions between the activated endothelium and circulating neutrophils [54].

Adherence to endothelial cells

The bacterial ligands that may be involved in adherence may include bundle-forming pili (see above) and several outer-membrane proteins (OMPs). Of the nine proteins located in the outer membrane of *B. henselae*, five were shown to bind human umbilical vein endothelial cells [55]. Of these, a 43-kDa protein (Omp43), that exhibits a similar amino acid sequence to the Omp2b porin of *Brucella* spp., was shown to have especially high affinity with endothelial cells, suggesting that it may play a major role in *Bartonella* pathogenesis [55, 56]. The endothelial receptors involved in *Bartonella* adhesion may include intercellular adhesion molecule-1 (ICAM-1), which has been shown to be enriched especially at the tips of the protrusions of the endothelial membrane [52]. Interestingly, endothelial adhesion molecules (ICAM-1 and E-selectin) expression is upregulated *via* NF-κB translocation, induced by *B. henselae* [52].

Invasion of endothelial cells

Endothelial cells are invaded by two mechanisms: (i) endocytosis of bacteria, similar to that present in other intracellular clades, and (ii) the engulfment of clustered bacteria by a unique host cell structure called the invasome [52]. Invasion is associated with cytoskeletal re-arrangements, themselves induced by *Bartonella via* Rho GTPase signalling [41].

Type IV secretion system and the VirB operon

Type IV secretion systems consist of a multiprotein channel that transports DNA or protein from bacteria to host cell. Such a system is present in *A. tumefaciens*, another alpha-2 proteobacterium that parasitises plants. A gene cluster, the *virB* operon of *A. tumefaciens,* plays a critical role in the formation of the channel through which the transfer of oncogenic T-DNA occurs, resulting in tumour formation [58]. Type IV secretion systems coded by the *virB* operon are also present in *Br. suis* and *Bordetella pertussis*, where they appear to be required for survival in host macrophages and exportation of the pertussis toxin, respectively [59, 60]. Recently, Padmalayan *et al*. discovered that the gene encoding an immunogenic 17-kDa antigen of *B. henselae* was located within the *virB* operon of *B. henselae* [61]. The *virB* operon of *B. henselae* encodes 10 genes, of which eight share significant homology to those of *A. tumefaciens*, suggesting that it encodes for a type IV secretion system. Interestingly, no homologues of the gene encoding the immunogenic 17-kDa antigen are present outside *Bartonella* spp. [61]. Its expression is stimulated by endothelial cells [62]. Although the molecule that may be transferred to the host cell remains to be defined, some have speculated that it may be involved in the production of VEGF by the endothelial cells.

Activation of endothelial cells

A pro-inflammatory activation of endothelial cells was postulated, which is thought to be a result of receptor-ligand interactions between the activated endothelium and circulating neutrophils [54]. This is supported by the recent demonstration that *B. henselae* itself and *B. henselae*-derived OMPs induce an NF-κB-dependent upregulation of E-selectin and ICAM-1 in endothelial cells, which in turn results in enhanced polymorphonuclear rolling and adhesion [57].

Primary niche

In the rat model of *B. tribochorum* infection, the presence of periodic erythrocyte infection waves has been demonstrated [9], that echo the 5-day periodicity of the ‘Quintan fever’. The fact that *Bartonella* parasitise erythrocytes without leading to haemolysis, with the exception of *B. bacilliformis*, suggests that the re-infection waves are due to the liberation of the bacteria from a distant sanctuary site [54]. This unknown primary niche might be the endothelial cells, as suggested by Dehio [54]. However, a number of hints suggest that the primary niche or sanctuary site might instead be located in the bone marrow, as follows. (i) Although, *in vitro*, *Bartonella* is able to enter various cells, including macrophages [63], only erythrocytes and endothelial cells are permissive to *Bartonella in vivo*. The cells that play the role of primary niche should thus share some characteristics common to erythrocytes and endothelial cells. Candidates are mainly the cells that are issued by differentiation of the haemangioblast, the common precursor of both erythrocytes and endothelial cells. These include mainly angioblasts and erythroblasts. (ii) The fact that erythroblasts express VEGF [49], a factor known to enhance *Bartonella* replication [38]. (iii) All organs involved in bacillary angiomatosis could potentially play the role of sanctuary, including brain, penis, vulva, cervix, muscle and bone marrow [64–68]. However, after skin, bone is the second most frequent site, and bone lesions of bacillary angiomatosis are characterised by well circumscribed osteolysis, that is often painful and usually affects long bones [69]. Pain pattern and osteolysis echo those found in mastocytosis and multiple myeloma, suggesting that bone marrow cells may be infected by *Bartonella*. (iv) The outstanding features of trench fever are pain and tenderness in the shins and relapsing fever [70]. Many subjects also present only with painful shins, which were often, in the absence of fever, wrongly attributed to flat feet or rheumatism due to prolonged standing in mud and water [70]. This suggests that *B. quintana* may involve the bone marrow in trench fever patients. (v) The fact that bone marrow cells are highly permissive to *Br. melitensis,* a phylogenetically close relative of *Bartonella* spp. and to many other intracellular pathogens.

Conclusions

Future research should especially be aimed at defining the factors (i) determining the host specificity (especially for human-specific species), (ii) involved in erythrocyte and endothelial cell infection, and (iii) controlling *Bartonella*-associated angiogenesis. Moreover, the location in the host of a *Bartonella* sanctuary, if any, which could be responsible for the observed relapses of intra-erythrocytic infections should be identified. Bone marrow progenitors such as erythroblasts might be a potential candidate but this should be investigated further.

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