Bartonella bacilliformis: Molecular Mechanisms of Invasion

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ABSTRACT

***Bartonella bacilliformis* causes Carrion's disease. It invades erythrocytes during the acute phase and endothelial cells during the chronic phase of this disease. Studies have shown that flagellin, ialAB, and deformin as well as other proteins are involved in erythrocyte invasion. Endothelial cell invasion has a different mechanism. It requires a 43 kDa antigen and the activation of Rho.**

INTRODUCTION

Bartonellosis, also called Carrion's disease, is a biphasic disease restricted to the South American Andes including the countries of Peru, Columbia, and Ecuador. This disease is caused by *Bartonella bacilliformis* infection, a gram-negative and haemotrophic bacterium, which enters the bloodstream of humans through the bite of a female sand fly, *Lutzomyia verrucarum* (Kreier and Ristic, 1981; Hertig, 1942). Only humans acquire bartonellosis. Bartonellosis causes two distinct clinical syndromes: Oroya fever and Verruga peruana. The Oroya fever is the primary, acute, and hematic phase characterized by fever, malaise, and a severe intravascular hemolytic anemia (Weinman, 1944; Reynafarje and Ramos, 1961; Garcia-Caceres and Garcia, 1991). In this phase, nearly 100% of erythrocytes are infected, and almost 80% of them are lysed (Weinman, 1944; Reynafarje and Ramos, 1961; Hutado et al., 1938). If untreated, this phase of disease has a 40% fatality rate (Weinman, 1944). Verruga peruana is the secondary, chronic, and tissue phase attributed to the invasion of endothelial cells by *B. bacilliformis.* Patients usually enter this phase following four to eight weeks of Oroya fever. Verruga peruana is characterized by angiomatous cutaneous eruptions (Gray et al., 1990). *B. bacilliformis* invades endothelial cells in this phase and subsequently stimulates the formation of new blood vessels and as well as endothelial cell proliferation (Garcia et al., 1990; Garcia et al., 1992; Hill et al., 1992).

ERYTHROCYTE INVASION

B. bacilliformis is a highly motile bacterium, with multiple flagella. The major component of the flagella, a 42 kDa protein called flagellin, was isolated and identified (Scherer et al., 1993). Anti-flagella antibody reduces binding of *B. bacilliformis* to erythrocytes, but cloned flagellin and flagella isolated directly from *B. bacilliformis* do not bind to erythrocytes directly (Scherer et al., 1993). This suggests that flagellin is indirectly involved in erythrocyte invasion.

Mature erythrocytes contain very little actin and are non-endocytotic. Treatment of erythrocytes with glycolysis and proton-motive-force inhibitors has no effect on *B. bacilliformis* adhesion (Walker and Winkler, 1981). Hence erythrocytes are passive and make no significant contribution to *B. bacilliformis* uptake or invasion.

In 1995, the *B. bacilliformis* invasion-associated locus A and B (ialAB) genes were identified. They code for a 20.1 kDa and 19.9 kDa protein, respectively (Mitchell and Minnick, 1995). IalAB were shown to be involved in erythrocyte invasion by conferring an erythrocyte-invasive phenotype upon non-invasive *E. coli* strains (Mitchell and Minnick, 1995). IalA is a (di)nucleoside polyphosphate hydrolase that hydrolyzes diadenosine and diguanosine (Cartwright el al., 1999; Conyers and Bessman, 1999). It is thought to be involved in reducing levels of stress-induced dinucleotides during invasion, thus aiding bacterial survival (Cartwright et al., 1999; Conyers and Bessman, 1999). Mutagenesis of ialB decreased bacterial association and invasion of human erythrocytes by 47 to 53%, while transcomplementation of ialB restored erythrocyte association and invasion rates to levels observed in the parental strain (Coleman and Minnick, 2001). These data provide direct evidence for the role of ialB as a virulence factor of *B. bacilliformis*. Moreover, they also showed that ialB is a membrane protein localized to the inner membrane of the pathogen (Coleman and Minnick, 2001).

An extracellular protein factor, deformin, was found in the supernatant of *B. bacilliformis* cultures (Mernaugh and Ihler, 1992). Deformin reproduced the morphological changes seen in *B. bacilliformis* infected erythrocytes, including the formation of pits, trenchs, and internal vacuoles (Mernaugh and Ihler, 1992). Deformin is 67 KDa but probably occurs as a dimer in its native state. In addition, deformin is sensitive to heat and protease treatment (Mernaugh and Ihler, 1992; Xu et al., 1995). Further study has revealed that deformin is actually a small water-soluble molecule, approximately 1.4 kDa in size that is bound tightly to albumin, especially albumin dimers and multimers present in the growth medium (Weinman, 1944).

Several other *B. bacilliformis* factors, which may be involved in the invasion of erythrocytes, were isolated

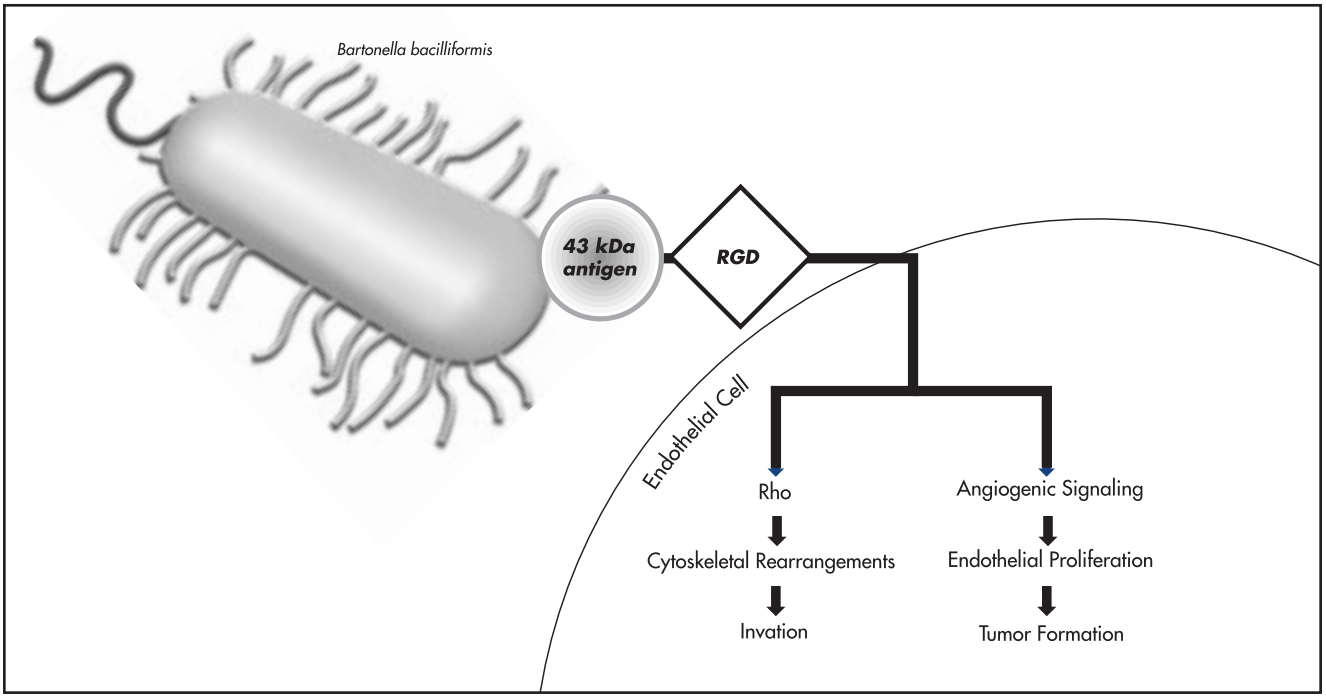

FIGURE 1 | Model of endothelial cell invasion by *Bartonella bacilliformis*. The 43 kDa antigen mediates the interaction between *B. bacilliformis* and endothelial cell via its arginine-glycine-aspartate (RGD) motif. This interaction may trigger the activation of Rho GTPases, which leads to the cytoskeletal rearrangements necessary for bacterial internalization. This interaction may also stimulate angiogenic signaling, which results in endothelial cell proliferation and tumor formation.

and studied. Minnick (1994) identified 14 *B. bacilliformis* outer membrane proteins (from 11.2 to 275.3 kDa) that may be involved in erythrocyte binding. A gene, which codes a 27.4 kDa protein and conferred the invasive phenotype on non-invasive *E. coli*, was isolated and sequenced in 1996 (Raji et al., 1996). In 1997, 6 *B. bacilliformis* membrane proteins that mediate the interaction with erythrocytes were identified, with sizes of 100, 92, 84, 46, and 12 kDa, respectively (Iwaki-Egawa and Ihler, 1997). Several other proteins were also identified, including α and β spectrin (230 and 210 kDa, respectively), band 3 protein (100 kDa), glycophorin A (83 kDa), and glycophorin B (44 kDa dimer and 25 kDa monomer) (Iwaki-Egawa and Ihler, 1997; Buckles and McGinnis Hill, 2000).

ENDOTHELIAL CELL INVASION

Endothelial cell invasion by *B. bacilliformis* is very different from erythrocyte invasion. Studies of endothelial cell invasion by *B. bacilliformis* demonstrate that endothelial cells actively participate in *bacterial* uptake. In addition endothelial cells are induced by *B. bacilliformis* to undergo cytoskeletal reconfiguration to enhance uptake (Hill et al., 1992). These data suggest that a surface-associated factor is involved in the endothelial cell invasion process and that internalization of the bacterium by host cell involves a microfilament-dependent process similar to phagocytosis (Hill et al., 1992). Rearrangement of the actin cytoskeletal network is partly controlled by the intracellular signaling protein, RhoA. Verma et al. (2000) studied the role of Rho in the invasion of endothelial cells by *B. bacilliformis*. They demonstrated that activation of Rho is required for internalization of *B. bacilliformis* and that *B. bacilliformis* activated intracellular Rho of the endothelial cells infected (Verma et al., 2000). Moreover, using both in vitro and in vivo systems it has been demonstrated that *B. bacilliformis* produces factors that stimulate endothelial cells to proliferate, release tissue plasminogen activator (t-PA, t-PA release is an in vitro characteristic of an angiogenic factors), and stimulate the formation of new blood vessels (Garcia et al., 1990). It was shown that these angiogenic factors are specific for endothelial cells, heat sensitive, and different from all other known angiogenic factors (Garcia et al., 1990).

By screening the *B. bacilliformis* genomic library with the serum of a patient in the Verruga peruana phase of Carrion's disease, an immunogenic 43 kDa *B. bacilliformis* antigen was cloned, sequenced, and characterized (Padmalayam et al., 2000). This 43 kDa antigen is a lipoprotein. There is a homologue of this 43 kDa antigen in *B. henselae*, a bacterium that is phylogenetically closely related to *B. bacilliformis* (Padmalayam et al., 2000; Ihler, 1996; Jerris and Regnery, 1996). Both *B. henselae* and *B. bacilliformis* can invade endothelial

cells and cause endothelial cell proliferation. However, *B. henselae* does not have the capacity to invade erythrocytes (Iwaki-Egawa and Ihler, 1997). A signal peptide cleavage site, a characteristic feature of lipoproteins that are exported to the outer membrane, was revealed in the 43 kDa *B. bacilliformis* antigen by amino acid sequence examination (Padmalayam et al., 2000; Yamaguchi et al., 1998). In addition, near the carboxyl-terminal end of the 43 kDa antigen is the tripeptide cell adhesion motif, arginine-glycine-aspartate (RGD) (Padmalayam et al., 2000; Buckley et al., 1999; Stockbauer et al., 1999). Therefore, this 43 kDa antigen may be critical for *B. bacilliformis* mediated endothelial cell proliferation and endothelial cell invasion (Figure 1).

CONCLUSION

In summary, proteins, like flagellin, ialAB, deformin, RhoA, and the 43 kDa antigen have begun to shed light on the molecular mechanisms by *B. bacilliformis* invasion. The functions of these proteins are still not fully understood, and many other factors involved in the invasion process have yet to be found. Interestingly, the same signal transduction pathways used by *B. bacilliformis* during invasion may also be involved in the tumorigenesis. Future studies would not only illuminate potential treatments for the chronic phase of Carrion's disease, but also provide important clues about the molecular mechanisms of cancer.

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