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"If you bring an alarm, we will destroy it," said *Brucella* to the host cell

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Pathogenic brucellae are the agent of brucellosis, a disease that causes abortion and infertility in natural hosts such as sheep, goats, or cattle. *Brucella melitensis, Brucella abortus*, and *Brucella suis* are the most pathogenic *Brucella* species for humans and also the most relevant bacteria involved in economical loses and in animal and human health problems. In addition, these *Brucella* represent a zoonotic risk in low-income countries.[1,2] Humans are infected through contacts with infected animals by aerosols or by ingestion of contaminated dairy products. Human brucellosis is a re-emerging febrile illness that may progress into a chronic phase characterized by the appearance of severe complications such as endocarditis, arthralgia, epididymitis, etc.[1,3] If untreated, chronic brucellosis represents a threat, especially in endemic areas (Central and South America, the Middle East, Mediterranean countries, northern Africa, and countries of the Caucasus and Central Asia).[4] A figure of 500 000 new cases/year is usually given as an estimate.[3] Because of all these circumstances, brucellosis is classified according WHO among the top seven neglected zoonoses. These diseases in endemic area are considered as a human health problem with a direct link to poverty.

Brucella species are facultative gram-negative intracellular bacteria. In both humans and animals, *Brucella* first targets the respiratory epithelium, the conjunctiva, and sexual organs. Even nowadays, the cells targeted by the pathogen for entry remain uncharacterized and efforts have to be done to decipher where and how *Brucella* invade the body. However, what we do know is that bacteria internalized by phagocytes at the periphery move to regional lymph nodes, which may play a barrier to subsequent systemic dissemination. *Brucella* is capable of colonizing macrophages, monocytes, and dendritic cells and can be found in large numbers in the liver, the medulla, and the spleen.[1] Therefore, it is not surprising that *Brucella* has engineered several devices to make its pathogenic life easier, defeating both innate and adaptive immunity. *Brucella*, like many other intracellular pathogenic bacteria, secretes effector proteins inside the host cytoplasm of infected cells in order to circumvent essential functions of the host defense, the final goal being the establishment a long-lasting chronic infection beneficial for the invader.

The mechanisms involved in *Brucella* entry into host cells still remain to be characterized. *Brucella* can colonize macrophages and dendritic cells (DCs) as well as trophoblasts, fibroblasts, endothelial cells, and epithelial cells.

In both murine macrophages and human monocytes *Brucella* enters through lipid rafts.[5,6] This event, also observed in DCs is dependent on the PI3-kinase and TLR4.[7-9] Indeed, it has been shown that *Brucella* mutants lacking LPS O-chain do not use lipid rafts and are killed by the host cell suggesting that the *Brucella* O-chain plays an important role in early events of the *Brucella*-containing vacuole (BCV).[10-12]

Brucella entry into host cells also depends on the expression of BvrR/BvrS. This two component regulator system controls the expression of genes controlling the acylation of the *Brucella* LPS lipid A and the surface expression of several outer membrane proteins.[13-15] Lipid raft-mediated *Brucella* internalization has been proposed to be under the control of the class A scavenger receptor[16] and the cellular prion protein PrPc[17] in macrophages. *Brucella* adhesion to macrophages and epithelial cells seems to be associated to the host surface expression of sialic acids, which bind the *Brucella* surface protein 41 encoded by the *ugpB* locus.[18] Other *Brucella* proteins such as the product of the gene BMEI0216 have been implicated in adhesion and/or internalization into phagocytes.[19] In addition, the *B. abortus efp* gene and the pathogenicity island Bab1_2009–2012 encoding a *Brucella* adhesin seem to play a role in *Brucella* uptake.[20,21]

In this issue of *Virulence*, Alva-Perez et al.[22] investigated the role of a subfamily of (di)nucleoside oligophosphate molecules linked to other "X" molecules (NUDIX) enzymes. NUDIX have been described in other bacteria as invasins and are present in *Brucella* spp. The authors called this NUDIX enzyme InvA and generated a deletion mutant of the *B. melitensis invA* gene to understand its role in virulence. Such a mutant was attenuated during the first steps of invasion in HeLa cells and goat macrophages with a maximum attenuation at 2 h p.i. Interestingly, the mutant strain exhibited a low level of colocalization with cathepsin D, similar to the parental strain colocalization at 24 h p.i. showing that the *invA* gene is important during invasion but not for intracellular replication. The authors also showed that InvA

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was important for survival in vivo in mice. The major point here is that *Brucella* needs to turn off the power of oxidative stress. Under stress, increasing intracellular concentrations of alarmones (oligophosphate nucleotides) are sensed as a danger signal by the cell, which in turn will be ready to prepare for a stress adaptation. To inhibit the toxic effects of alarmone accumulation, bacterial NUDIX enzymes can hydrolyze alarmones, thereby promoting invasion and intracellular survival. This new mechanism of host response subversion highlights the adaptation of intracellular bacteria to their environment.

When BCVs are formed by interaction with early endosomes, they then loose early endosome markers and concomitantly acquire late endosomal/lysosomal membrane proteins such as LAMP1 and the small GTPase Rab7.[23,24] Finally BCVs fuse with the endoplasmic reticulum as schematized in **Figure 1**.

The *Brucella* cyclic β-1,2-glucan (CβG) located in the periplasm of the bacteria, when released out of the bacterium is capable of modifying the composition of lipid rafts at the level of the BCV.[25] In macrophages but not in DCs, CβG is a virulence factor.[26] However, in contrast to other molecules expressed by *Brucella* such as the BtpA and BtpB, CβG appears to be a factor that mediates both macrophage and dendritic cell activation.[27] In the case of *Brucella* spp. a TIR domain containing protein called BtpA/TcpB controls Toll-like receptor (TLR) signaling.[26,28] BtpA is expressed by *B. abortus* and *B. melitensis* but seems to be absent from *B. suis*. BtpA was shown to interfere with TLR4 and TLR2 signaling through Myd88 interaction.[26,28] Other reports proposed that BtpA targets the adaptor protein MAL/TIRAP.[29,30] Direct comparison of the in vitro interaction between BtpA and either MyD88 or TIRAP showed a stronger interaction with MyD88.[31] BtpA has been shown to bind phosphoinositides at the plasma membrane[30] but also to induce ubiquitination of TIRAP.[29] Another Btp family member was recently discovered, namely BtpB.[32] BtpB is also translocated into host cell cytoplasm and interferes with the activation of dendritic cells. In vivo mouse studies revealed that BtpB contributes to virulence by controlling inflammatory responses. Together, *Brucella* TIR-containing proteins BtpA and BtpB modulate host inflammatory responses during infection.

Acidification of BCVs is an essential step for *Brucella* intracellular survival[33] and intracellular trafficking to the endoplasmic reticulum (ER).[24] Acidic pH in BCVs promotes the expression of several genes required for virulence. This the case for the *virB* operon that encodes a type IV secretion system (T4SS) involved in the secretion of *Brucella* effector proteins.[33]

Getting to the proximity of the replication niche, namely the ER, the intermediate compartment is a target for *Brucella*. Communicating between the Golgi apparatus and the small GTPase Rab2 seems to be a host target and required for *Brucella* intracellular multiplication.[34] Rab2 forms a complex of proteins with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the coat COPI complex and the protein kinase C (PKCλ). This complex controls the vesicular trafficking from the Golgi to the ER, via a vesicle/tubule cluster.[35] Each protein of the GAPDH/COPI/Rab2/PKCλ complex is required for *Brucella* intracellular replication. This suggests that BCVs interact first with the vesicle/tubule cluster before reaching the ER. Recently, the *Brucella* translocated RicA effector protein was identified and characterized to recruit the small GTPase Rab2. This important finding highlighted the interaction at the molecular level between a *Brucella* protein (RicA) and a host protein complex controlled by a small GTPase (Rab2), thereby showing how pathogens subvert host intracellular trafficking.[36]

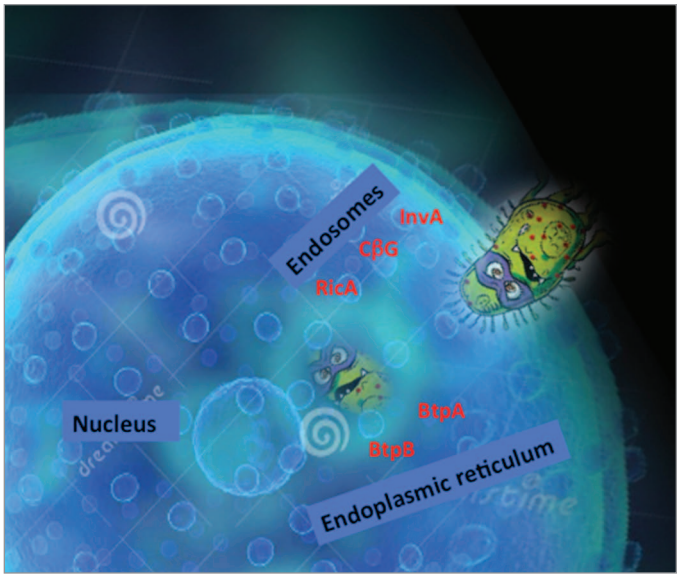

Figure 1. A schematic view of *Brucella* entry into a cell. *Brucella* uses different effector proteins that help the bacterium to travel from endosomes to finally hide inside the endoplasmic reticulum. We hypothesize that InvA protein acts at the level of entry during the first step of *Brucella* intracellular trafficking, followed by the action of the cyclic glucan (CbG), then RicA between late endosomes and the endoplasmic reticulum, and finally in the endoplasmic reticulum with BtpA and BtpB controlling host cell signaling.

Following still uncharacterized membrane fusion events with the secretory pathway that require the small GTPase Sar1 at the level of ER exit sites[37] BCVs finally fuse with the ER, a safe niche for *Brucella* replication.

Altogether, these results summarizing almost 20 years of research show that *Brucella* actually uses different angles to tackle host cell response and we are probably far away to know them all.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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