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The Interaction Between *Brucella* and the Host Cell in Phagocytosis

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Abstract

Brucella spp. are facultative intracellular parasitic pathogens that can survive and multiply in professional and nonprofessional phagocytes. These pathogens are responsible for brucellosis, which can cause abortion in domestic animals and undulant fever in humans. *Brucella* spp. can survive in a variety of cells and their virulence and chronic infections are thought to be due to their ability to evade the killing mechanisms within host cells, one of which is the inhibition of phagosome-lysosome fusion. Lipid raft-associated molecules, such as GPI-anchored proteins, GM1 ganglioside, and cholesterol, are selectively integrated into *Brucella*-containing macropinosomes following the internalization of *Brucella* into macrophages, continuously sustaining a dynamic state of the phagosomal membrane. Toll-like receptors (TLRs) are important systems that detect microbial invasion via recognition of microbial components that triggers signaling pathways to promote the expression of genes and regulate innate immune responses. Recent several studies have revealed the importance between TLRs-*Brucella* interactions to control *Brucella* infection. Here, we reviewed selected aspects of lipid raft-associated molecules and TLRs-*Brucella* interaction, which may help to understand the mechanism of *Brucella* pathogenesis.

Keywords: *Brucella*, phagocytes, lipid-rafts associated molecules, TLRs, intracellular survival

1. Introduction

Brucellosis is a major zoonotic disease worldwide that causes a serious debilitating disorder in humans known as undulant fever, and abortion and sterility in domestic animals.

Brucella spp. are gram-negative and facultative intracellular bacteria that can survive and replicate within professional and nonprofessional phagocytes [1, 2]. Six well-recognized species of *Brucella* are known according to host preference: *B. melitensis* (sheep and goats), *B. abortus* (cattle), *B. suis* (hogs), *B. ovis* (sheep), *B. canis* (dogs), and *B. neotomae* (wood rats) [3]. In the past few years, *Brucella* has been recovered from several marine mammals, including cetaceans and pinnipeds, that belong to two potential new species, *B. pinnipedialis* and *B. ceti* [4]. Recently, a new species of *Brucella*, *B. microti*, was isolated from wild common voles suffering from a systemic disease [5, 6]. *B. melitensis*, *B. abortus*, and *B. suis* strains cause abortion and infertility in their natural hosts, goats and sheep, cattle and swine, respectively. Humans can also acquire Brucellosis in a form of a severe, debilitating febrile illness as a result of contact with infected animals or their products [7]. *B. ovis* is a natural pathogen of sheep where it primarily causes epididymitis and infertility in rams [8].

B. canis infection causes abortion and infertility in dogs [9]. Although *B. ovis* and *B. canis* are important in animals, human infection with *B. canis* is rare [10], and human infection with *B. ovis* has not been reported. *B. neotomae*, which infects only desert wood rats, is not known to be associated with clinical disease in any host species.

Brucella species, in contrast to other intracellular pathogens, do not produce exotoxins, antiphagocytic capsules or thick cell walls, resistance forms, or fimbriae and do not show antigenic variation [11]. The key aspect of the virulence of *Brucella* is thought to be due to their ability to avoid the killing mechanisms within macrophages [12, 13].

The most common points of entry of *Brucella* are the respiratory, digestive, and genital tracts of both animals and humans. *Brucella* enters the phagocytic cells in an unknown cellular site and spreads throughout the body by the regional lymph nodes. *Brucella* shows high tropism in macrophages, especially monocytes in the liver, spleen, mammary glands, and reproductive tracts. Chronic brucellosis mainly leads to bacterial resistance to host immune response and host debilitated health status [14].

A tenth of the total *Brucella* will survive to avoid phagocytosis and penetrate cell membrane for intracellular growth; macrophages are the most important for a successful infection. During the infection, *Brucella* can interfere with the macrophage function, particularly the inhibition of IFN- γ [15] and TNF- α expression [16], and the reduction of antigen presentation and subsequent T cell activation [17]. *Brucella* inside dendritic cells (DC) contributes to the chronic infection and induced low levels of pro-inflammatory cytokines and increased MHC II expression [18]. Placental trophoblasts produce erythritol during the last trimester and increases carbon source for *Brucella* and this pathogen caused abortion or stillbirth of the infected fetus by inducing placental damage [19] and targeting giant trophoblasts [20]. *Brucella* has also been reported in other cell types and are studied with cell models and lines such as human pulmonary epithelial cells, caprine uterine epithelial cells, human osteoblastic cell lines, murine neurons, bovine and human polymorphonuclear, and many other cells lines. Surprisingly, extracellular brucellae were observed on the 21st day post infection [21–26].

Phagocytosis is a critical step for a successful immune reaction against microbial pathogens that provokes both degradation of pathogens and the subsequent presentation of pathogen

peptide antigens. Ligation of various phagocytic receptors, including Fc gamma receptors and complement receptor 3, activates a series of intracellular signal transductions that induce dynamic and rapid rearrangement of the actin cytoskeleton essential for phagocytic uptake [27]. Several host cells such as M cells, macrophages, and neutrophils ingest *Brucella* by zipper-like phagocytosis [28]. In addition, *Brucella* invades macrophages through lipid raft microdomains [29]. Phagocytosis of *Brucella* in both epithelial cells and macrophages requires F-actin polymerization [30, 31].

Toll-like receptors (TLRs) are the best characterized pattern recognition receptors (PRRs) of host cells. Receptor-ligand interaction via TLRs leads to the production of antimicrobial peptides and proinflammatory cytokines through NF- κ B, mitogen-activated protein kinase (MAPK), and other various signaling pathways [32]. As a result, TLR signaling is crucial to develop host innate immune response, including recruitment of DCs and T effector cells, upregulation of MHC I and II on antigen presenting cells (APCs), and extension of adaptive immunity against infection. In Brucellosis, many studies have reported that TLRs play important roles in controlling *Brucella* infection. When unopsonized *B. melitensis*, *B. abortus*, and *B. suis* strains internalize into macrophages and epithelial cells, the *Brucella*-containing vacuoles (BCVs) enter into an intracellular trafficking pathway that results in the development of specialized membrane-bound compartments [33–38] known as replicative phagosomes or brucellosomes [39]. Interactions between the O-chain of *Brucella* smooth LPS and the lipid rafts on the surface of macrophages have been shown to be important for mediating entry into host cells in a manner that leads to the development of replicative phagosome [40]. During the initial stages of intracellular trafficking of the BCVs, these compartments suffer temporary interactions with lysosomes [41] which results in their acidification [34, 42] and initiate extensive interaction with the endoplasmic reticulum [33]. Eventually, intracellular pH rises to a level that allows intracellular replication of the *Brucella*. In epithelial cells, the BCVs during development of the replicative phagosome acquire properties resembling autophagosomes [37], which does not appear to be the case in macrophages [33]. Studies employing the human monocytic cell line THP-1 and *B. abortus* strains opsonized with hyperimmune IgG have also shown that when the *Brucella* internalizes host macrophages in this manner, the resulting BCVs also undergo temporary association with the lysosomal compartment and become acidified but do not interact extensively with the ER [43]. This altered intracellular trafficking limits the fusion of the BCVs with lysosomes, which minimizes the exposure of these bacteria to the bactericidal proteins that reside in these intracellular compartments [43].

In this section, we will discuss the key roles of several receptors for *Brucella* including immune response, signal transduction cascade, and phagocytic pathway for *Brucella* infection within host cells.

2. The roles of lipid rafts on *Brucella* infection

Brucella proliferates within professional and nonprofessional phagocytic host cells including macrophages, epitheloid HeLa cells, fibroblasts NIH3T3, Vero cells, MDBK cells, etc., and

successfully bypasses the bactericidal effects of phagocytes [13]. The macrophage response to infection has important consequences for both the survival of phagocytized bacteria and the further development of host immunity. For many bacterial pathogens, adherence to the host tissue is believed to be essential for virulence, and the microbial characteristics that promote adherence to receptors on a host cell surface are considered to be attributes of virulence [44]. For intracellular pathogens, including *Brucella*, the nature of the interaction with the host cell will have important consequences for pathogen survival, proliferation, and dissemination, as well as the development of specific immunity [45]. Lipid rafts are specialized membrane microdomains rich in cholesterol, glycosylphosphatidylinositol (GPI)-anchored proteins, and GM1 gangliosides [46]. Evidence regarding the potential role of lipid rafts in host-pathogen interactions has been continuously accumulated, and lipid rafts have been implicated as portals of entry for intracellular pathogens [47]. Several studies have implicated the involvement of lipid rafts in the entry and endocytic pathway of *B. abortus* in host cells. These studies indicated that lipid raft-associated molecules, such as GPI-anchored proteins, GM1 ganglioside, and cholesterol, are selectively integrated into *Brucella*-containing macropinosomes following the internalization of *Brucella* into macrophages, continuously sustaining a dynamic state of the phagosomal membrane [48]. Moreover, the internalization route of *Brucella* into phagocytic cells determines the intracellular fate of these bacteria, and this event is modulated by lipid rafts [48].

2.1. Roles of lipid rafts-associated molecules in *Brucella* infection

Time-lapse videomicroscopy has been used to follow the internalization of *B. abortus* strains by mouse bone marrow-derived macrophages [35]. After contact of macrophages with wild-type *B. abortus*, the bacteria move around from the site of initial contact and swim on the macrophage surface, which often lasts up to several minutes; ruffling of the generalized plasma membrane occurs before the eventual enclosure in large vacuoles. In contrast, contact of the virB4 mutant of *B. abortus* with the target macrophage results in a much smaller ruffling restricted to the area near the bacteria and uptake is more rapid than for the wild-type strain. If the bacteria are deposited onto macrophages by centrifugation, generalized actin polymerization around the site of bacterial binding was observed in the wild-type strain when stained with phalloidin to detect actin filament formation by using fluorescence microscopy, which can also be observed by phase-contrast microscopy and the virB4 mutant shows primarily small regions of phalloidin staining at the sites of binding. Therefore, *B. abortus* appears to promote events on the macrophage cell surface that are dependent on the presence of the VirB system. In case of *B. abortus*, macropinocytosis occurs within minutes of attachment to bacteria on the surface of the macrophage. During bacterial contact, effector molecule(s) are translocated by the VirB system to the target cell, which initiates the process that leads to formation of the macropinosome [29, 35]. These macropinosomes are induced transiently and shrink rapidly, with the majority of vacuoles appearing tightly apposed against the bacterial surface within 20 minutes after their initial appearance. In addition, macropinosomes are probed with other components associated with lipid raft-associated molecules, such as GM1 gangliosides and cholesterol, by incubating *B. abortus* and biotin-labeled cholera toxin B subunit (CTB), which binds GM1-gangliosides, simultaneously with macrophages. CTB localizes around the

internalized wild-type strain with kinetics of association similar to those for aerolysin-labeled GPI-anchored proteins. In contrast, colocalization of CTB with the virB4 mutant was much less pronounced, suggesting that the formation of the VirB-dependent macropinosome includes a sorting process that allows transient association of lipid raft-associated components with macropinosomes containing *B. abortus*.

2.2. Roles of cellular prion protein in *Brucella* infection

In addition to membrane sorting for brucella infection, key roles have been made in describing bacterial entry where it has been shown that these bacteria penetrate into the macrophage through a particular structure found in eukaryotic cells, lipid rafts, or lipid microdomains [48]. In order to interact with lipid rafts, *Brucella* requires smooth LPS to avoid the bactericidal arsenal of macrophages that strains with rough LPS (without an O-side chain) encounter [40]. Moreover, a report has proposed that *Brucella* interacts with the cellular prion protein of macrophages (Prp^C), a protein anchored by a GPI-link in lipid rafts. This interaction was found to be mediated by the membrane expression of *Brucella* HSP60 [49].

2.3. Roles of clathrin in *Brucella* infection

Lipid raft-associated clathrin is essential for host-pathogen interactions in infectious processes. The focus of a recent study was to elucidate the clathrin-mediated phagocytic mechanisms of *Brucella* [50]. From that study, the clathrin dependency of *Brucella* infection in HeLa cells was investigated with an infection assay and immunofluorescence microscopy. The redistribution of clathrin in the membrane and phagosomes was detected through sucrose gradient fractions of lipid rafts and the isolation of *Brucella*-containing vacuoles (BCVs), respectively (Fig. 1). Clathrin and dynamin were concentrated into lipid rafts upon *Brucella* infection, and the entry and intracellular survival of *Brucella* were abrogated by clathrin inhibition in HeLa cells. Clathrin disruption decreased actin polymerization and the colocalization of BCVs with clathrin and Rab5 but not LAMP-1. Consequently, our data verified that clathrin plays a fundamental role in the entry and intracellular survival of *Brucella* via the interaction with lipid rafts and actin rearrangement, which determines the early intracellular trafficking of *Brucella* to its advantage.

3. General aspects of toll-like receptors

Toll-like receptors (TLRs) are single-pass type I transmembrane-spanning proteins with a single intracellular Toll/interleukin-1 (IL-1) receptor (TIR) domain and multiple extracellular leucine-rich repeats (LRRs) responsible for binding to ligands that recognize and are activated by a small collection of microbe-derived molecules [51]. Through studies of targeted mutants among 13 paralogous TLRs, 10 in humans and 12 in mice, the diverse mode of ligands recognition of individual TLRs were determined, except for TLR8, TLR10 (only present in humans), and TLR11–13 (only present in mice). TLR2 is activated by lipopeptides and other gram-positive bacterial components in conjunction with either TLR1 or TLR6; TLR4 detects

LPS, which requires accessory protein MD-2; TLR5 detects flagellin; TLR3 detects poly I:C, a double-stranded RNA (dsRNA) analog; TLR9 detects unmethylated DNA and CpG-oligo-deoxynucleotides (CpG-DNA) proposed to be delivered by Granulin and high mobility group (HMG) B proteins through an ability to bind simultaneously to both CpG-DNA and TLR9; and TLR7 is activated by single-stranded RNA and its synthetic analogs such as resiquimod, imiquimod, and loxoribine. All known TLR dimer structures display the same arrangement with the two carboxy-terminal tails closely juxtaposed and the amino termini at opposite ends but each varies in modes of ligand recognition [51–54]. This conformation may be required to bring the intracellular TIR domains into close proximity to initiate signaling. TLR activation can induce cell-intrinsic antimicrobial activity such as activation of TLR2 and TLR4 can recruit NADPH oxidase assembly and mitochondria to bacteria-containing phagosome, which lead to a burst of reactive oxygen and nitrogen species within this compartment [55–57]. Evidence suggests that possibly through recruitment of vacuolar-ATPase subunits to the phagosomal membrane, TLR signaling can cause a rapid acidification of the phagosome in which TLR signaling has occurred [53, 54, 58, 59]. These activities increase the antimicrobial capacity of the phagosome, although some bacteria have actually cooped these signals to regulate their virulence programs. Expression and secretion of antimicrobial peptides (AMPs) such as β -defensins and cathelicidin can also be induced by TLRs upon detection of microbial ligands, which further supports the role of TLR-mediated detection in cell-intrinsic antimicrobial activity [60–62]. However, pathogens have evolved a variety of strategies to avoid TLR signaling such as altering their surface structures, interfering with TLR signaling pathways, and inhibiting, escaping, or subverting phagocytosis [52]. *Brucella* spp. are recognized by TLR2, TLR4, and TLR9, which identifies lipopolysaccharide (LPS), lipoproteins, and bacterial DNA, respectively [63].

3.1. TLRs and *Brucella* infection

The involvement of TLR2 and TLR4 in recognizing *Brucella* was reported in several studies. TLR2 was proposed to induce secretion of TNF- α , IL-6, IL-12, and IL-10 in peritoneal macrophages stimulated by *B. abortus* lipoproteins, such as Omp16 and Omp19 [64], responsible for pro-inflammatory response, but no role was observed in controlling the pathogen in vivo [63]. TLR4, in cooperation with TLR9, was demonstrated in *B. melitensis* resistance [65]. The interaction of TLR4 with non-canonical *Brucella* LPS induces activation of NF- κ B, and its interaction with *Brucella* spp. lumazine synthase stimulates maturation of dendritic cells [66] followed by increased expression of co-stimulatory molecules and major histocompatibility class II, as well as the production of IL-6, TNF- α , and IL-12p70 [63].

Maturation of dendritic cells and production of IL-12 and TNF- α in macrophages and dendritic cells are impaired [67], and levels of inflammatory chemokines RANTES (CCL5), MCP-1 (CCL2) and MIP-1 α (CCL) are reduced in the absence of MyD88 protein during *Brucella* infection [65]. MyD88 molecule is required for the development of IFN- γ producing T cells and control of brucellosis [65], suggesting that induction of Th1 response during the infection is regulated by a MyD88-dependent pathway [63]. Furthermore, this molecule is used by other

inflammatory signaling pathways that include IL-1 and IL-18 [58]. However, IL-18 was observed to have no role in controlling murine brucellosis [63].

Brucella appears to interfere in TLR signaling by producing inhibitory homologues of Toll/interleukin-1 receptor (TIR) domain, such as *B. abortus* Btp1, which targets TLR2 signaling down-modulating maturation of infected dendritic cells and secretion of pro-inflammatory cytokines [18], and *B. melitensis* TcpB that interacts with MyD88 in vitro impeding TLR2 and TLR4 activation pathway and secretion of pro-inflammatory cytokines [68].

3.2. Roles of individual TLRs in *Brucella* infection

3.2.1. TLR2

The role of TLR2 in *Brucella* infection remains controversial. Some studies suggest that TLR2 is not required to control *Brucella* infection in the mouse [67, 69, 70]. However, other studies indicate that TLR2 is important for clearance of *Brucella* from the lung following aerosol exposure [71], cytokine production such as TNF α and IL-12 [64, 65, 67, 72, 73], MHC-II expression [74], and down regulation of the type I receptor for the Fc portion of IgG (Fc γ RI, CD64) [15].

3.2.2. TLR4

The role of TLR4 in *Brucella* infection also remains disputed. Some studies suggest that TLR4 is required to control *Brucella* replication in the mouse [65, 69, 70], others reveal that TLR4 is not involved [67, 75]. Lee et al. [76] reported that TLR4-associated Janus kinase 2 (JAK2) activation in the early cellular signaling events plays an essential role in *B. abortus*-induced phagocytosis by macrophages (Fig. 2), implying the significance of JAK2 in pathogenesis of *Brucella* [65]. TLR2, TLR4, and MyD88 play diverse roles in *Brucella* antigen specific antibody production and antibody class switching [71].

3.3. TLR6

TLR6 is an important component that triggers an innate immune response against *B. abortus*. TLR6 is recruited to the macrophage phagosome and recognizes bacterial peptidoglycan and lipoproteins [77]. TLR6 also plays a role in bacterial diacylated lipopeptides recognition such as MALP2, but is not essential for cytokine production in response to triacylated lipopeptides. TLR6, in cooperation with TLR2, recognizes *Brucella* and further activates NF- κ B signaling in vitro and is required for the efficient control of *B. abortus* infection in vivo [78].

3.4. TLR9

TLR9 plays a role in controlling *B. abortus* infection in mice [65, 67]. Furthermore, TLR9 partially mediates the expression of IL-12 by dendritic cells in response to heat-killed *B. abortus* [79]. TLR9 plays a significant role in preventing *B. ovis* replication in vivo, but only MyD88 is required for wild type levels of inflammation [80].

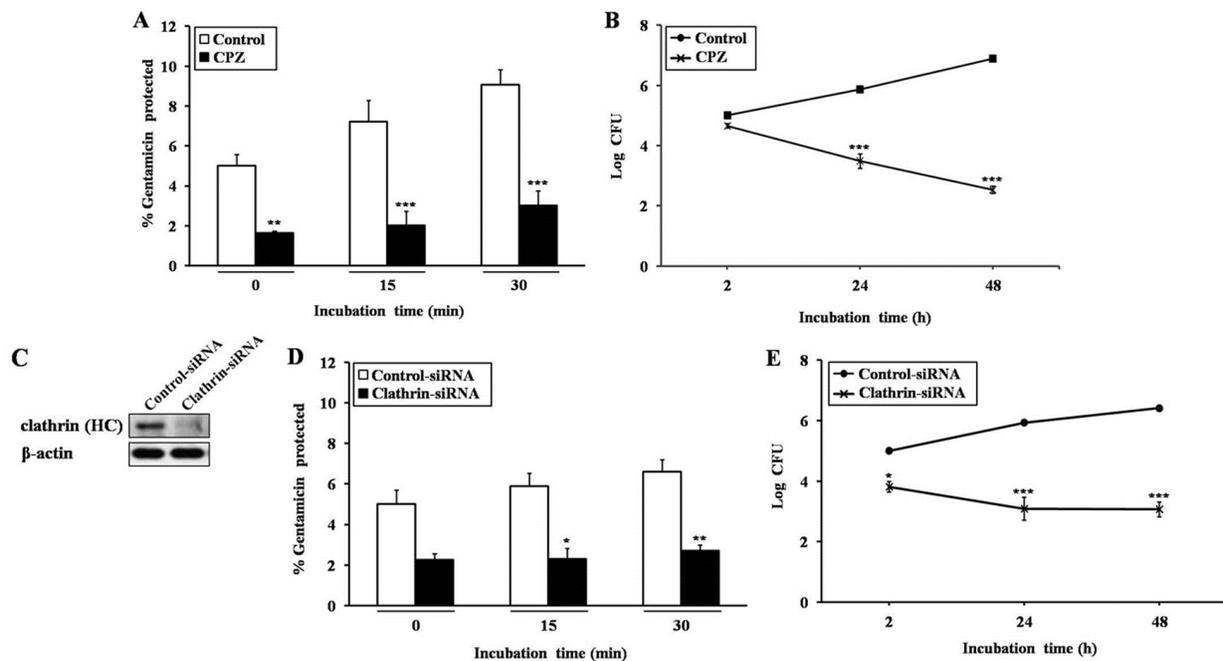


Figure 1. The role of clathrin in the entry and intracellular survival of *B. abortus* in non-professional phagocytes. A and B: HeLa cells were pretreated with 12.5 μ M CPZ, a clathrin inhibitor, for 45 minutes prior to infection with *B. abortus* at an MOI of 10 for the indicated times. C–E: HeLa cells were transiently transfected with control or clathrin siRNA, whose optimal conditions were evaluated by Western blotting (C), and subsequently infected according to the procedure described above (D and E). Bacterial internalization and intracellular survival efficiency were determined by evaluating the protection of internalized bacteria from gentamicin killing and calculating the log₁₀ CFU, respectively. The data represent the mean \pm S.D. of triplicate trials from three independent experiments. Differences that were statistically significant compared with untreated samples are indicated. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ [50].

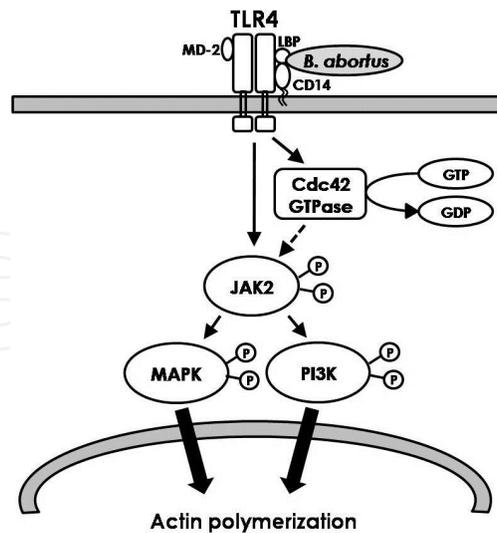


Figure 2. Diagram illustrating the phagocytic signaling pathway initiated by TLR4-linked JAK2 activation during the internalization of *B. abortus* into macrophage. The interaction of *B. abortus* with TLR4 induces the activation of Cdc42 GTPase and JAK2, and the subsequent activation of PI3K and MAPKs promotes actin polymerization. This event contributes to the phagocytosis of *B. abortus* by macrophage. Lines with arrows denote an activating reaction and dotted lines denote uncertainty of the reaction [76].

4. Conclusion

Throughout this chapter, we described the interaction between *Brucella* and lipid rafts-associated molecules and TLRs, including interacting specific molecules (ligands), immune response, signal cascade, and controlling strategies. This review may help to understand the pathogenic and defense mechanisms of Brucellosis. Furthermore, the understanding of lipid rafts-associated molecules and TLRs-mediated controlling of intracellular parasitic bacterial infection would be helpful to eradicate these diseases.

Abbreviation

IFN- γ	Interferon gamma
TNF- α	Tumor necrosis factor alpha
DC	Dendritic cell
MHC II	Major histocompatibility complex
TLR	Toll-like receptor
PRRs	Pattern recognition receptors
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
MAPK	Mitogen-activated protein kinase
APCs	Antigen-presenting cells
BCVs	<i>Brucella</i> -containing vacuoles
LPS	Lipopolysaccharide
IgG	Immunoglobulin
ER	Endoplasmic reticulum
GPI	Glycosylphosphatidylinositol
GM1	Monosialotetrahexosylganglioside
CTB	Cholera toxin B
Prp ^C	Cellular prion protein
HSP60	Heat shock protein 60
LAMP-1	Lysosomal-associated membrane protein 1
IL	Intracellular Toll/interleukin
TIR	Intracellular Toll/interleukin receptor
LRRs	Leucine-rich repeats

HMG	High mobility group
NADPH	Nicotinamide adenine dinucleotide phosphate
AMPs	Antimicrobial peptides
Omp	Outer membrane protein
RANTES	Regulated on activation, normal T cell expressed and secreted
MCP-1	Monocyte chemotactic protein 1
MIP-1 α	Macrophage inflammatory protein 1 alpha
Th	T helper
JAK2	Janus kinase 2
MALP-2	Macrophage-activating lipopeptide-2

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References

- [1] Detilleux PG, Deyoe BL, Cheville NF. Entry and intracellular localization of *Brucella* spp. in Vero cells: Fluorescence and electron microscopy. *Vet Pathol.* 1990; 27: 317-328.
- [2] Detilleux PG, Deyoe BL, Cheville NF. Penetration and intracellular growth of *Brucella abortus* in nonphagocytic cells in vitro. *Infect Immun.* 1990; 58: 2320-2328.
- [3] Ko J, Splitter GA. Molecular host-pathogen interaction in brucellosis: Current understanding and future approaches to vaccine development for mice and humans. *Clin Microbiol Rev.* 2003; 16: 65-78.
- [4] Foster G, Osterman BS, Godfroid J, Jacques I, Cloeckert A. *Brucella ceti* sp. nov. and *Brucella pinnipedialis* sp. nov. for *Brucella* strains with cetaceans and seals as their preferred hosts. *Int J Syst Evol Microbiol.* 2007; 57: 2688-2693.

- [5] Hubalek Z, Scholz HC, Sedlacek I, Melzer F, Sanogo YO, Nesvadbova J. Brucellosis of the common vole (*Microtus arvalis*). *Vector Borne Zoonotic Dis.* 2007; 7: 679-687.
- [6] Scholz HC, Hubalek Z, Sedlacek I, Vergnaud G, Tomaso H, Al Dahouk S, Melzer F, Kampfer P, Neubauer H, Cloeckert A, Maquart M, Zygmunt MS, Whatmore AM, Falsen E, Bahn P, Gollner C, Pfeffer M, Huber B, Busse HJ, Nockler K. *Brucella microti* sp. nov., isolated from the common vole *Microtus arvalis*. *Int J Syst Evol Microbiol.* 2008; 58: 375-382.
- [7] Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. *Lancet Infect Dis.* 2006; 6: 91-99.
- [8] Buckrell BC, McEwen SA, Johnson WH, Savage NC. Epididymitis Caused by *Brucella ovis* in a Southern Ontario Sheep Flock. *Can Vet J.* 1985; 26: 293-296.
- [9] Wanke MM. Canine brucellosis. *Anim Reprod Sci.* 2004; 82: 195-207.
- [10] Corbel MJ. Brucellosis: An overview. *Emerg Infect Dis.* 1997; 3: 213-221.
- [11] Finlay BB, Falkow S. Common themes in microbial pathogenicity revisited. *Microbiol Mol Biol Rev.* 1997; 61: 136-169.
- [12] Sangari FJ, Aguero J. Molecular basis of *Brucella* pathogenicity: An update. *Microbiologia.* 1996; 12: 207-218.
- [13] Ugalde RA. Intracellular lifestyle of *Brucella* spp. Common genes with other animal pathogens, plant pathogens, and endosymbionts. *Microbes Infect.* 1999; 1: 1211-1219.
- [14] Martirosyan A, Moreno E, Gorvel JP. An evolutionary strategy for a stealthy intracellular *Brucella* pathogen. *Immunol Rev.* 2011; 240: 211-234.
- [15] Barrionuevo P, Delpino MV, Velasquez LN, Garcia Samartino C, Coria LM, Ibanez AE, Rodriguez ME, Cassataro J, Giambartolomei GH. *Brucella abortus* inhibits IFN-gamma-induced Fc gamma RI expression and Fc gamma RI-restricted phagocytosis via toll-like receptor 2 on human monocytes/macrophages. *Microbes Infect.* 2011; 13: 239-250.
- [16] Caron E, Gross A, Liautard JP, Dornand J. *Brucella* species release a specific, protease-sensitive, inhibitor of TNF-alpha expression, active on human macrophage-like cells. *J Immunol.* 1996; 156: 2885-2893.
- [17] Forestier C, Deleuil F, Lapaque N, Moreno E, Gorvel JP. *Brucella abortus* lipopolysaccharide in murine peritoneal macrophages acts as a down-regulator of T cell activation. *J Immunol.* 2000; 165: 5202-5210.
- [18] Salcedo SP, Marchesini MI, Lelouard H, Fugier E, Jolly G, Balor S, Muller A, Lapaque N, Demaria O, Alexopoulou L, Comerci DJ, Ugalde RA, Pierre P, Gorvel JP. *Brucella* control of dendritic cell maturation is dependent on the TIR-containing protein Btp1. *PLoS Pathog.* 2008; 4: e21.

- [19] Tobias L, Cordes DO, Schurig GG. Placental pathology of the pregnant mouse inoculated with *Brucella abortus* strain 2308. *Vet Pathol.* 1993; 30: 119-129.
- [20] Kim S, Lee DS, Watanabe K, Furuoka H, Suzuki H, Watarai M. Interferon-gamma promotes abortion due to *Brucella* infection in pregnant mice. *BMC Microbiol.* 2005; 5: 22.
- [21] Ferrero MC, Fossati CA, Baldi PC. Smooth *Brucella* strains invade and replicate in human lung epithelial cells without inducing cell death. *Microbes Infect.* 2009; 11: 476-483.
- [22] Meador VP, Hagemoser WA, Deyoe BL. Histopathologic findings in *Brucella abortus*-infected, pregnant goats. *Am J Vet Res.* 1988; 49: 274-280.
- [23] Delpino MV, Fossati CA, Baldi PC. Proinflammatory response of human osteoblastic cell lines and osteoblast-monocyte interaction upon infection with *Brucella* spp. *Infect Immun.* 2009; 77: 984-995.
- [24] Garcia Samartino C, Delpino MV, Pott Godoy C, Di Genaro MS, Pasquevich KA, Zwerdling A, Barrionuevo P, Mathieu P, Cassataro J, Pitossi F, Giambartolomei GH. *Brucella abortus* induces the secretion of proinflammatory mediators from glial cells leading to astrocyte apoptosis. *Am J Pathol.* 2010; 176: 1323-1338.
- [25] Iyankan L, Singh DK. The effect of *Brucella abortus* on hydrogen peroxide and nitric oxide production by bovine polymorphonuclear cells. *Vet Res Commun.* 2002; 26: 93-102.
- [26] Rolan HG, Xavier MN, Santos RL, Tsolis RM. Natural antibody contributes to host defense against an attenuated *Brucella abortus* virB mutant. *Infect Immun.* 2009; 77: 3004-3013.
- [27] Gruenheid S, Finlay BB. Microbial pathogenesis and cytoskeletal function. *Nature.* 2003; 422: 775-781.
- [28] Ackermann MR, Cheville NF, Deyoe BL. Bovine ileal dome lymphoepithelial cells: Endocytosis and transport of *Brucella abortus* strain 19. *Vet Pathol.* 1998; 25: 28-35.
- [29] Watarai M, Makino S, Fujii Y, Okamoto K, Shirahata T. Modulation of *Brucella*-induced macropinocytosis by lipid rafts mediates intracellular replication. *Cell Microbiol.* 2002; 4: 341-355.
- [30] Guzman-Verri C, Chaves-Olarte E, von Eichel-Streiber C, Lopez-Goni I, Thelestam M, Arvidson S, Gorvel JP, Moreno E. GTPases of the Rho subfamily are required for *Brucella abortus* internalization in nonprofessional phagocytes: Direct activation of Cdc42. *J Biol Chem.* 2001; 276: 44435-44443.
- [31] Kusumawati A, Cazevielle C, Porte F, Bettache S, Liautard JP, Sri Widada J. Early events and implication of F-actin and annexin I associated structures in the phagocyt-

ic uptake of *Brucella suis* by the J-774A.1 murine cell line and human monocytes. *Microb Pathog.* 2003; 28: 343-352.

- [32] Kawai T, Akira S. TLR signaling. *Cell Death Differ.* 2006; 13: 816-825.
- [33] Celli J, de Chastellier C, Franchini DM, Pizarro-Cerda J, Moreno E, Gorvel JP. *Brucella* evades macrophage killing via VirB-dependent sustained interactions with the endoplasmic reticulum. *J Exp Med.* 2003; 198: 545-556.
- [34] Arenas GN, Staskevich AS, Aballay A, Mayorga LS. Intracellular trafficking of *Brucella abortus* in J774 macrophages. *Infect Immun.* 2000; 68: 4255-4263.
- [35] Kim S, Watarai M, Makino S, Shirahata T. Membrane sorting during swimming internalization of *Brucella* is required for phagosome trafficking decisions. *Microb Pathog.* 2002; 33: 225-237.
- [36] Naroeni A, Jouy N, Ouahrani-Bettache S, Liautard JP, Porte F. *Brucella suis*-impaired specific recognition of phagosomes by lysosomes due to phagosomal membrane modifications. *Infect Immun.* 2001; 69: 486-493.
- [37] Pizarro-Cerda J, Meresse S, Parton RG, van der Goot G, Sola-Landa A, Lopez-Goni I, Moreno E, Gorvel JP. *Brucella abortus* transits through the autophagic pathway and replicates in the endoplasmic reticulum of nonprofessional phagocytes. *Infect Immun.* 1998; 66: 5711-5724.
- [38] Rittig MG, Alvarez-Martinez MT, Porte F, Liautard JP, Rouot B. Intracellular survival of *Brucella* spp. in human monocytes involves conventional uptake but special phagosomes. *Infect Immun.* 2001; 69: 3995-4006.
- [39] Kohler S, Michaux-Charachon S, Porte F, Ramuz M, Liautard JP. What is the nature of the replicative niche of a stealthy bug named *Brucella*? *Trends Microbiol.* 2003; 11: 215-219.
- [40] F, Naroeni A, Ouahrani-Bettache S, Liautard JP. Role of the *Brucella suis* lipopolysaccharide O antigen in phagosomal genesis and in inhibition of phagosome-lysosome fusion in murine macrophages. *Infect Immun.* 2003; 71: 1481-1490.
- [41] Starr T, Ng TW, Wehrly TD, Knodler LA, Celli J. *Brucella* intracellular replication requires trafficking through the late endosomal/lysosomal compartment. *Traffic* 2008; 9: 678-694.
- [42] Porte F, Liautard JP, Kohler S. Early acidification of phagosomes containing *Brucella suis* is essential for intracellular survival in murine macrophages. *Infect Immun.* 1999; 67: 4041-4047.
- [43] Bellaire BH, Roop RM, 2nd, Cardelli JA. Opsonized virulent *Brucella abortus* replicates within nonacidic, endoplasmic reticulum-negative, LAMP-1-positive phagosomes in human monocytes. *Infect Immun.* 2005; 73: 3702-3713.

- [44] Casadevall A, Pirofski L. Host-pathogen interactions: The attributes of virulence. *J Infect Dis.* 2001;1884: 337-344.
- [45] Jimenez de Bagues MP, Dudal S, Dornand J, Gross A. Cellular bioterrorism: How *Brucella* corrupts macrophage physiology to promote invasion and proliferation. *Clin Immunol.* 2005;114: 227-238.
- [46] Brown DA, London E. Functions of lipid rafts in biological membranes. *Annu Rev Cell Dev Biol.* 1998; 14: 111-136
- [47] Gatfield J, Pieters J. Essential role for cholesterol in entry of mycobacteria into macrophages. *Science* 2000; 288: 1647-1650.
- [48] Naroeni A, Porte F. Role of cholesterol and the ganglioside GM(1) in entry and short-term survival of *Brucella suis* in murine macrophages. *Infect Immun.* 2002; 70: 1640-1644.
- [49] Watarai M, Kim S, Erdenebaatar J, Makino S, Horiuchi M, Shirahata T, Sakaguchi S, Katamine S. Cellular prion protein promotes *Brucella* infection into macrophages. *J Exp Med.* 2003; 198: 5-17.
- [50] Lee JJ, Kim DG, Kim DH, Simborio HL, Min W, Lee HJ, Her M, Jung SC, Watarai M, Kim S. Interplay between clathrin and Rab5 controls the early phagocytic trafficking and intracellular survival of *Brucella abortus* within HeLa cells. *J Biol Chem.* 2013; 288: 28049-28057.
- [51] Moresco EM, LaVine D, Beutler B. Toll-like receptors. *Curr Biol.* 2011; 21: 488-493.
- [52] Arpaia N, Barton GM. The impact of Toll-like receptors on bacterial virulence strategies. *Curr Opin Microbiol.* 2013; 16: 17-22.
- [53] Blander JM, Medzhitov R. Regulation of phagosome maturation by signals from toll-like receptors. *Science* 2004; 304: 1014-1018.
- [54] Blander JM, Medzhitov R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature.* 2006; 440: 808-812.
- [55] Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol.* 2004; 4: 181-189.
- [56] Underhill DM, Ozinsky A. Phagocytosis of microbes: Complexity in action. *Annu Rev Immunol.* 2002; 20: 825-852.
- [57] West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, Walsh MC, Choi Y, Shadel GS, Ghosh S. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature.* 2011; 472: 476-480.
- [58] Arpaia N, Godec J, Lau L, Sivick KE, McLaughlin LM, Jones MB, Dracheva T, Peterson SN, Monack DM, Barton GM. TLR signaling is required for *Salmonella typhimurium* virulence. *Cell.* 2011; 144: 675-688.

- [59] Trombetta ES, Ebersold M, Garrett W, Pypaert M, Mellman I. Activation of lysosomal function during dendritic cell maturation. *Science*. 2003; 299: 1400-1403.
- [60] Redfern RL, Reins RY, McDermott AM. Toll-like receptor activation modulates antimicrobial peptide expression by ocular surface cells. *Exp Eye Res*. 2011; 92: 209-220.
- [61] Rodriguez-Martinez S, Cancino-Diaz ME, Cancino-Diaz JC. Expression of CRAMP via PGN-TLR-2 and of alpha-defensin-3 via CpG-ODN-TLR-9 in corneal fibroblasts. *Br J Ophthalmol*. 2006; 90: 378-382.
- [62] Tauszig S, Jouanguy E, Hoffmann JA, Imler JL. Toll-related receptors and the control of antimicrobial peptide expression in *Drosophila*. *Proc Natl Acad Sci*. 2000; 97: 10520-10525.
- [63] Oliveira SC, de Oliveira FS, Macedo GC, de Almeida LA, Carvalho NB. The role of innate immune receptors in the control of *Brucella abortus* infection: Toll-like receptors and beyond. *Microbes Infect*. 2008; 10: 1005-1009.
- [64] Giambartolomei GH, Zwerdling A, Cassataro J, Bruno L, Fossati CA, Philipp MT. Lipoproteins, not lipopolysaccharide, are the key mediators of the proinflammatory response elicited by heat-killed *Brucella abortus*. *J Immunol*. 2004; 173: 4635-4642.
- [65] Macedo GC, Magnani DM, Carvalho NB, Bruna-Romero O, Gazzinelli RT, Oliveira SC. Central role of MyD88-dependent dendritic cell maturation and proinflammatory cytokine production to control *Brucella abortus* infection. *J Immunol*. 2008; 180: 1080-1087.
- [66] Berguer PM, Mundinano J, Piazzon I, Goldbaum FA. A polymeric bacterial protein activates dendritic cells via TLR4. *J Immunol*. 2006; 176: 2366-2372.
- [67] Weiss DS, Takeda K, Akira S, Zychlinsky A, Moreno E. MyD88, but not toll-like receptors 4 and 2, is required for efficient clearance of *Brucella abortus*. *Infect Immun*. 2005; 73: 5137-5143.
- [68] Cirl C, Wieser A, Yadav M, Duerr S, Schubert S, Fischer H, Stappert D, Wantia N, Rodriguez N, Wagner H, Svanborg C, Miethke T. Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain-containing proteins. *Nat Med*. 2008; 14: 399-406.
- [69] Campos MA, Rosinha GM, Almeida IC, Salgueiro XS, Jarvis BW, Splitter GA, Qureshi N, Bruna-Romero O, Gazzinelli RT, Oliveira SC. Role of Toll-like receptor 4 in induction of cell-mediated immunity and resistance to *Brucella abortus* infection in mice. *Infect Immun*. 2004; 72: 176-186.
- [70] Copin R, De Baetselier P, Carlier Y, Letesson JJ, Muraille E. MyD88-dependent activation of B220-CD11b+LY-6C+ dendritic cells during *Brucella melitensis* infection. *J Immunol*. 2007; 178: 5182-5191.

- [71] Pei J, Ding X, Fan Y, Rice-Ficht A, Ficht TA. Toll-like receptors are critical for clearance of *Brucella* and play different roles in development of adaptive immunity following aerosol challenge in mice. *Front Cell Infect Microbiol.* 2012; 2: 115.
- [72] Huang LY, Aliberti J, Leifer CA, Segal DM, Sher A, Golenbock DT, Golding B. Heat-killed *Brucella abortus* induces TNF and IL-12p40 by distinct MyD88-dependent pathways: TNF, unlike IL-12p40 secretion, is Toll-like receptor 2 dependent. *J Immunol.* 2003; 171: 1441-1446.
- [73] Zwerdling A, Delpino MV, Barrionuevo P, Cassataro J, Pasquevich KA, Garcia Samartino C, Fossati CA, Giambartolomei GH. *Brucella* lipoproteins mimic dendritic cell maturation induced by *Brucella abortus*. *Microbes Infect.* 2008; 10: 1346-1354.
- [74] Barrionuevo P, Cassataro J, Delpino MV, Zwerdling A, Pasquevich KA, Garcia Samartino C, Wallach JC, Fossati CA, Giambartolomei GH. *Brucella abortus* inhibits major histocompatibility complex class II expression and antigen processing through interleukin-6 secretion via Toll-like receptor 2. *Infect Immun.* 2008; 76: 250-262.
- [75] Barquero-Calvo E, Chaves-Olarte E, Weiss DS, Guzman-Verri C, Chacon-Diaz C, Rucavado A, Moriyon I, Moreno E. *Brucella abortus* uses a stealthy strategy to avoid activation of the innate immune system during the onset of infection. *PLoS One* 2007; 2: e631.
- [76] Lee JJ, Kim DH, Kim DG, Lee HJ, Min W, Rhee MH, Cho JY, Watarai M, Kim S. Toll-like receptor 4-linked Janus kinase 2 signaling contributes to internalization of *Brucella abortus* by macrophages. *Infect Immun.* 2013; 81:2448-2458.
- [77] Ozinsky A, Underhill DM, Fontenot JD, Hajjar AM, Smith KD, Wilson CB, Schroeder L, Aderem A. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc Natl Acad Sci.* 2000; 97:13766-13771.
- [78] de Almeida LA, Macedo GC, Marinho FA, Gomes MT, Corsetti PP, Silva AM, Cassataro J, Giambartolomei GH, Oliveira SC. Toll-like receptor 6 plays an important role in host innate resistance to *Brucella abortus* infection in mice. *Infect Immun.* 2013; 81:1654-1662.
- [79] Huang LY, Ishii KJ, Akira S, Aliberti J, Golding B. Th1-like cytokine induction by heat-killed *Brucella abortus* is dependent on triggering of TLR9. *J Immunol.* 2005; 175: 3964-3970.
- [80] Vieira AL, Silva TM, Mol JP, Oliveira SC, Santos RL, Paixao TA. MyD88 and TLR9 are required for early control of *Brucella ovis* infection in mice. *Res Vet Sci.* 2013; 94: 399-405.