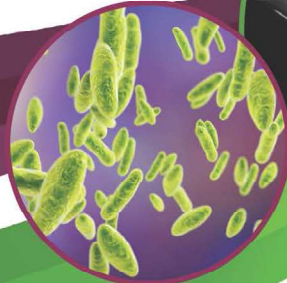


Brucellosis in Goats and Sheep

An Endemic and Re-Emerging
Old Zoonosis in the 21st Century

João Simões
Maria José Saavedra
Pamela A. Hunter
Editors

Animal Science,
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**JOÃO CARLOS CAETANO SIMÕES
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Chapter 5

PATHOGENESIS OF *BRUCELLA*

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ABSTRACT

The goal of this chapter is to describe the pathogenesis of *Brucella* reporting, the host-pathogen and the cell/macrophage *Brucella* interactions, and the major virulence factors of this bacteria genus. The epithelium of the respiratory, digestive and reproductive tracts are the most important ports of bacterium entry in the host. The mechanisms by which *Brucella* enters the cells and evades the host immune system remains poorly understood. However, in the past decade, the mechanisms of *Brucella* pathogenesis and host immunity have been extensively investigated. *Brucella* has the ability to survive and replicate intracellularly in mononuclear phagocytes and to control host immune responses. This pathogen developed several strategies to evade the host's immune defense mechanisms preventing inflammatory responses at the site of entrance in the host and maintain the infection. *Brucella* bacterium is internalized by macrophages and dendritic cells, and invades the bloodstream and lymphatics causing an eventual transitory bacteremia. Inside phagocytic cells, few bacteria can survive. *Brucella* also shows a strong tissue tropism to monocytes in the liver, spleen, lymph nodes, bone marrow and trophoblasts. All these target cells lead to clinical manifestations, characterized by infection in lymphoid tissues and inflammatory lesions in the reproductive tract of pregnant females. Unlike other bacteria, *Brucella* lacks classical bacterial virulence factors such as exotoxins, capsules, secreted proteases, fimbriae, flagella, virulence plasmids, resistant strains and phage-encoded toxins. Other virulence factors have been implicated in the pathogenesis as the type IV secretion system, the BvrR/BvrS two component regulatory system, the *Brucella* intact lipopolysaccharide O-antigen in smooth strains, and cyclic β -1,2-glucans. Toll-like receptors are single-pass type I transmembrane-spanning of proteins that play a key role in the innate immune system. Future perspectives include new genomics and omics technologies and software tools to analyze virulent genes associated with a *Brucella* infection. This information will provide a better knowledge of infection and *Brucella*-host relations that are necessary for vaccine production and strategies to prevent and control brucellosis in small ruminants.

Keywords: *Brucella*, pathogenesis, macrophage, type IV secretion system, two component regulatory system, β -1,2-glucans, toll-like receptors, virulence factors

INTRODUCTION

Brucella is a facultative, intracellular bacterium that controls the host immune system in order to establish chronic infection. In the past decade, the mechanisms of *Brucella* pathogenesis and host immunity have been extensively investigated. However, the mechanisms by which *Brucella* enters the cells and evades the host immune system remains poorly understood (He, 2012).

Brucella pathogenicity resides mainly in its ability to survive and replicate intracellularly in mononuclear phagocytes and to control host immune responses. This microorganism developed several strategies to dodge the host's immune defense mechanisms at the site of entrance and maintain infection, such as escaping intracellular destruction and limiting exposure to innate and adaptive immune responses or sequesters the pathogen. *Brucella* is a weak inducer of the host inflammatory response (Rolán et al., 2009; Ahmed et al., 2016). Pathogenesis and lesion development in small ruminants infected with *B. melitensis* are similar to that of cattle infected with *B. abortus* (Aparicio, 2013).

Pathogenesis mechanisms of *Brucella* includes *virB* operon which encodes the type IV secretion system (T4SS) (O'Callaghan et al., 2012; Ke et al., 2015), the BvrR/BvrS two component regulatory system encoded by *bvrR* and *bvrS* genes (Sola-Landa et al., 1998; Martínez-Núñez et al., 2010), the *Brucella* intact LPS O-antigen in smooth strains, and cyclic β -1,2-glucans (Lapaque et al., 2006). These virulence factors play an important role in intracellular survival process of the pathogen inside compartments of macrophages (Xiang et al., 2006).

Brucella has developed several strategies to establish and maintain an infection, including evasion of autophagic recognition, prevention of lysosomal fusion, inhibition of apoptosis of infected cells, interference of

dendritic cells (DCs) maturation and establishment of an endoplasmic reticulum (ER)-derived/decorated compartment (Adams, 2002; Radomsky et al., 2017).

Inbred mice, particularly BALB/c and C57BL strains, have been used most frequently as laboratory models for studying the pathogenesis of brucellosis. However, murine models may be more closely related to human infection where splenomegaly is a common clinical observation. Most studies used intra-peritoneal infection, but aerosol, oral, and intranasal routes have also been used. Other animal models that have been used include guinea pigs, rabbits, rats, and goats. Bacterial loads in the spleen and/or liver are most commonly used to assess pathogenesis. However, in preferred hosts, *Brucella* spp. localize more frequently in lymphatic tissues, supramammary lymph nodes, and reproductive organs (Elzer et al., 2002; Jiménez de Bagüés et al., 2010; Hanna et al., 2011; Olsen and Palmer, 2014).

This chapter addresses the host-pathogen and cell/macrophage-*Brucella* interactions as well as describes the major virulence factors of this bacteria genus.

HOST-PATHOGEN INTERACTIONS

Although significant advances have been made in recent years, the mechanisms that allow host invasion by *Brucella* spp. are poorly understood (Rossetti et al., 2017). *Brucella* spp. enters their hosts through contact with infected animals or organic material such as blood or milk or through aerosol (OIE, 2008). The most important ports of entry in hosts are the respiratory tract (nasal mucosae), the reproductive tract and digestive tract (oral mucosae). The digestive tract is the main port of entry for *B. abortus* and *B. melitensis*, while the reproductive tract is for *B. canis* and *B. ovis*. *Brucella* spp. can also enter mammalian hosts through skin abrasions or cuts, the conjunctiva, and copula (Alton and Forsyth, 1996; Sidell et al., 1997; Rossetti et al., 2013).

This genus has molecular determinants that allow them to invade, resist intracellular killing and reach their replicating niche in phagocytic and non-phagocytic cells. *Brucella* spp. display strong tissue tropism and replicate within vacuoles in macrophages, DCs, and placental trophoblasts. However, in *in vitro* assays the pathogen has the ability to replicate in a wide variety of mammalian cell types, including microglia, fibroblasts, epithelial cells, and endothelial cells (Moreno and Moriyó, 2006; García Samartino et al., 2010; Hamer et al., 2014).

The bacteria invade the bloodstream and lymphatics where they multiply inside phagocytic cells and eventually cause bacteremia (Kojouri and Gholami, 2009).

Brucella shows strong tropism and replicates within macrophages (especially), monocytes in the liver and spleen, DCs and trophoblasts which represent the major target cells for the pathogen leading to clinical manifestations, characterized by infection in lymphoid tissues and inflammatory lesions in the reproductive tract of pregnant ruminants (Ficht, 2003; Billard et al., 2005; Xavier, 2009).

In the gastrointestinal tract, *Brucella* spp. are phagocytosed by lymphoepithelial and commonly it is within neutrophils in the intestinal lumen and mononuclear phagocytes between dome lymphoepithelial cells. In the lamina propria of the dome, brucellae were present inside neutrophils, mononuclear phagocytes, or free in the interstitium, cells of gut-associated lymphoid tissue, from which they gain access to the submucosa. Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them, and are also phagocytosed by macrophages. Bacteria transported in macrophages, which travel to lymphoid tissue draining the infection site, may eventually localize in lymph nodes, liver, spleen, mammary gland, joints, kidneys, and bone marrow (Grilló et al., 2012).

The first bacteraemia occurs after invasion and *Brucella* migrates, transported by macrophages and accumulates in liver and lymphoid tissues such as the spleen and the iliac, mesenteric and supra mammary lymph nodes, where they may induce a granulomatous reaction. Bacteraemia promotes *Brucella* dissemination to different internal body organs and

tissues as in the milk, blood, semen, lymph nodes, reproductive tissues, aborted fetuses, arthritis, and hygroma fluids (Çiftci et al., 2017). A second bacteraemia results in generalized infection affecting other target organs, such as the pregnant uterus and the udder, as well as their associated lymph nodes. If the animal is pregnant when infection occurs, *Brucella* localize in pregnant uterus during the first bacteriemia (Ficht, 2003; Billard et al., 2005; Xavier, 2009).

Replication of the bacteria in placental trophoblasts during the late phase of gestation in ruminants results in placentitis, infection of the foetus and abortion (Carvalho Neta et al., 2008). Placental trophoblasts produce erythritol during the last trimester. *Brucella* preferentially utilized this sugar as a carbon source (Keppie et al., 1965; Barbier et al., 2017).

Extracellular growth occurs in the presence of erythritol (Petersen et al., 2013). It is possible that erythritol a four-carbon sugar, or the catabolic enzyme aldose reductase (Barbier et al. 2017) plays a fundamental role and justifies at least partially the *Brucella* trophism to genital organs. High concentrations of erythritol have shown to stimulate the growth of *B. melitensis* (Keppie et al., 1965; Petersen et al., 2013).

CELL/MACROPHAGE *BRUCELLA* INTERACTION

Brucella infects hosts primarily by adhering and penetrating mucosal epithelium surface receptors that contain sialic acid and sulfate residues (Rossetti et al., 2012). After entering, *Brucella* translocates through the mucosal barrier, invades epithelial cells and is capable of surviving intracellularly within phagocytic and nonphagocytic host cells as trophoblasts, epithelioid HeLa cells, fibroblasts, NIH3T3, vero cells, MDBK (Madin-Darby Bovine Kidney) cells, etc. (Anderson and Cheville, 1986; Kim, 2015). Macrophages, neutrophils and M (Microfold) cells ingest *Brucella* by zipper-like mechanisms of internalization (Ackermann et al., 1988; Pei et al., 2008). *Brucella* has the ability to invade macrophages via cholesterol-rich lipid rafts (Kim et al., 2002; Watarai et al., 2002).

In *in vitro* assays, virulent *Brucella* has an initial adaptation period followed by an intracellular replicative phase inside epithelial cells (Rossetti et al., 2012).

Brucella has developed a stealthy strategy to reach its replication niche and to escape recognition of the innate immunity through PAMPs (pathogen-associated molecular patterns) reduction, modification and hiding. This strategy ensuring low stimulatory activity and toxicity for cells (Barquero-Calvo et al., 2007).

Internalization

Brucella spp. is internalized by macrophages and DCs (Huang et al., 2001). Inside phagocytic cells less than 10% of phagocytized bacteria survive after an adaptation period (de Figueiredo et al., 2015). Smooth *Brucella* strains prevent macrophage cell death, however, rough attenuated *Brucella* strains, which lack the O-antigen, cannot survive inside macrophages and often induce programmed macrophage cell death (Chen et al., 2011; Li and He, 2012). DCs are professional antigen presenting cells critical for bridging innate and adaptive immune responses and are required for cytokine secretion (Li and He, 2012). Internalization of *Brucella* into host cells invasion implicates activation of regulatory proteins and cytoskeletal modifications (Pizarro-Cerdá et al., 1999; Guzmán-Verri et al., 2001). Mediators of cell signaling pathways such as cyclic GMP (Guanosine Monophosphate), PI3-kinase (Phosphoinositide 3-kinase), tyrosine protein kinases and mitogen-activated protein kinases (MAP) also are involved in internalization acting as second messengers for signals from the GTPases (hydrolase enzymes that can bind and hydrolyze guanosine triphosphate) (Guzmán-Verri et al., 2001; Chaves-Olarte et al., 2002).

After internalization, the majority of phagocytosed *Brucella* is destroyed by bactericidal activation of free radicals of oxygen, nitric oxide, and enzymes inside phagolysosomes. The *Brucella*-containing vacuole (BCV) fuse rapidly with the lysosome and can actively exclude lysosomal

proteins, and redirect the BCV to the endoplasmic reticulum where the organism is capable of replicating (Pizarro-Cerdá et al., 1998; Celli et al., 2003; Starr et al., 2008).

Toll-like receptors (TLRs) are single-pass type I transmembrane-spanning of proteins that play a key role in the innate immune system. TLRs receptors are localized on the plasma membrane (e.g., TLR4) or on endosomal membrane compartments (e.g., TLR9), and recognize PAMPs (Pathogen-Associated Molecular Patterns) such as lipoproteins, LPS, flagellin, or nucleic acids (Jang et al., 2015).

TLRs are important systems that increase antimicrobial capacity and detect microbial invasion (West et al., 2011) via recognition of microbial components that trigger signaling pathways to promote the expression of genes and regulate innate immune responses (Takeda and Akira, 2001; Kim, 2015).

TLRs regulate downstream cytokine expression by interacting and activating signaling pathways such as MyD88 (Myeloid Differentiation Primary-Response Protein 88) and TRIF (TIR-domain-containing adapter-inducing interferon- β). Those signaling pathways lead to activation of NF- κ B protein (Nuclear Factor Kappa-light-chain-enhancer of activated B cells), which is a cytoplasmic transcription factor that initiates transcription of a wide range of genes involved in the inflammatory response including cytokines, chemokines, and immunoreceptors (Kabelitz et al., 2006). Some TLRs, as TLR2, TLR4 and TLR9 have been implicated in host interactions with *Brucella*. This TLRs initiate limited intracellular signaling that activates the transcription factor (NF- κ B protein) to control expression of inflammatory cytokine genes (Oliveira et al., 2008).

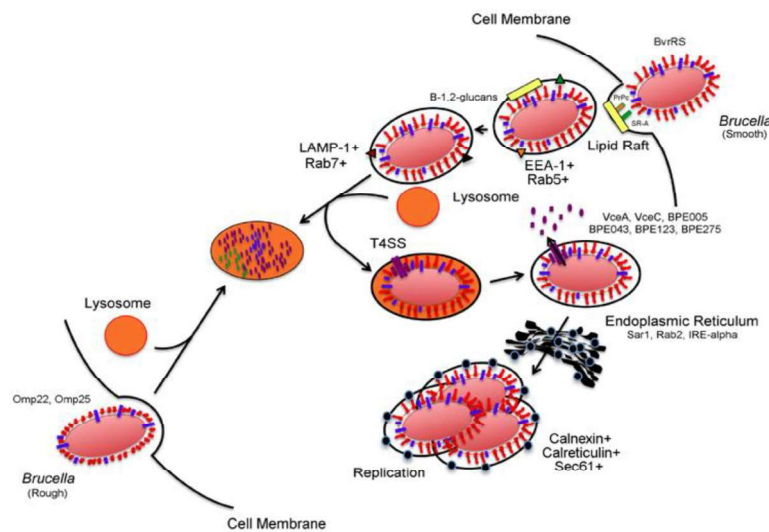
TLR4 is crucial for the detection of LPS, which is present in the cell wall of Gram-negative bacteria (Agnese et al., 2002). TLR4 plays a role in resistance to *B. abortus* infection. TLR2 and TLR4 signaling during *Brucella* infection are mediated by two different ligands, lipoproteins and LPS, respectively (Cardoso et al., 2006). *Brucella* spp. are recognized by TLR2 and TLR4 and TLR9, which identifies LPS, lipoproteins and bacterial DNA, respectively (Li et al., 2014). *Brucellae* has a LPS with weak endotoxic properties, which weakly stimulate TLR of the innate

immune system in sentinel cells of mucosa surfaces (Heumann and Roger, 2002).

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 (Pei et al., 2012; Kim, 2015).

Survival and Intracellular Trafficking

B. melitensis inhibits transcription of various host genes involved in apoptosis and intracellular vesicular trafficking (Figure 5.1; Eskra, 2003; He, 2012).



Legend: Smooth *Brucella* is internalized in a vacuole which fuses with host cell lysosomes for replication-competent bacteria mediated by several major virulence factors (lipopolysaccharide O-antigen; β -1,2-glucans T4SS: type 4 secretion system; and others: see the description of these factors in next pages). In contrast, rough *Brucella* suffer lysosomal degradation with Omp22 and Omp25 involvement.

Figure 5.1. Intracellular trafficking in host mammalian cells after *Brucella* cell invasion (Gomez et al., 2013).

Macrophages are the preferred cells infected by *Brucella*. *Brucella* strains with smooth LPS enter macrophages through interaction with lipid rafts and are then encompassed in BCV. This vacuole retains some lipid raft markers, targeting the BCV to the ER. *Brucella* fuses with the ER, thus acquiring ER markers to avoid fusion with the lysosome before beginning to replicate. Rough LPS mutants do not enter the macrophage through lipid rafts and are rapidly targeted to the lysosome and killed (Haag et al., 2010).

Inside mononuclear phagocytic cells, brucellae has the ability to control and modify the intracellular trafficking. *Brucella* resides in a special vacuole, called the BCV to avoid its degradation. *Brucella* transforms the vacuole into a replicative compartment. The microorganism adapts soon after invasion at the microenvironment inside BCV with limited nutrient availability (Pizarro-Cerdá et al., 2008; Starr et al., 2008; He, 2012).

After sustained interaction and fusion with endoplasmic reticulum (ER), mature BCVs become replicative compartments (i.e., replicative paghosomes) with ER-like properties. This late maturation event requires a functional T4SS (Celli et al., 2003). The T4SS of brucellae support maturation of the BCV and ultimately controls the intracellular and stealthy lifestyle of the pathogen (Döhmer et al., 2014). Virulent *Brucella* successfully fused with ER cisternae and survived and multiplied. However, attenuated *Brucella* failed to fuse with ER and bacteria were destroyed inside of the host phagolysosomes (Lamontagne et al., 2009).

Brucellae avoids intracellular destruction by restricting fusion of the BCV with the lysosomal compartment which results in their acidification (Pizarro-Cerdá et al., 1998). Altered intracellular trafficking limits the fusion of BCVs with lysosomes which minimizes the exposure of these bacteria to bactericidal proteins that reside in these intracellular compartments. Following this process, the BCV becomes associated with the endoplasmic reticulum-associated compartments which are the niches for intracellular replication of brucellae in macrophages and can establish chronic infection (Pizarro-Cerdá et al., 1998; Celli et al., 2003).

MAJOR VIRULENCE FACTORS

The mechanisms that regulate expression of virulence genes is still not entirely understood. *Brucella* is a pathogen that lacks classical bacterial virulence factors, such as exotoxins, cytolisins, capsules, secreted proteases, fimbriae, flagella, virulence plasmids, lysogenic phages, resitant strains, phage-encoded toxins, endotoxic LPS and induces host cells apoptosis (Seleem et al., 2008; He, 2012; Rossetti et al., 2017).

However, other virulence factors have been identified, such as LPS, VirB type IV secretion system (VirB T4SS), the BvrS/BvrR two-component regulatory system (BvrS/BvrR TCS), and cyclic β -1,2-glucan (C β G). Among the various brucellae systems and molecules known to participate as virulence factors, the BvrR/BvrS TCS and the VirB T4SS are critical (Martínez-Núñez et al., 2010).

The virulence mechanisms identified until now are those required for host cell invasion and escape mechanisms for intracellular survival or replication (Xavier, 2010).

VirB Type IV Secretion System

T4SS is one of several types of secretion systems that are extensively used by intravacuolar Gram-negative bacteria to colonize host cells and for the induction of the hosts' immune response (Mitchell et al., 2016).

Expression of brucellae T4SS acts as a virulence factor. T4SS, encoded by the VirB operon located in chromosome II, is required for invasion and early intracellular survival, replication, phagosome maturation and regulation of intracellular trafficking of the *Brucella*-containing vacuole, in phagocytic and non-phagocytic cells (O'Callaghan et al., 1999; den Hartigh et al., 2008; Xavier, 2010; Ke et al., 2015). T4SS seems to be essential for prolonged persistence by recruitment cells that contribute to persistence (de Figueiredo et al., 2015). It is possible that is also required for brucellae to reach its intracellular replication niche (Derakhshandeh et al., 2013; Xavier, 2010; Poester et al., 2013).

T4SS is responsible for the delivery of effector proteins into host cell cytosol, that control the intracellular and stealthy lifestyle of the pathogen (Voth et al., 2012; Smith et al., 2016). T4SS is also necessary to induce B cell maturation, TCD4⁺ cell (mature T-helper cells) activation and initial secretion of IL-12 (Interleukin-12) and interferon gamma (IFN- γ) (Rólan and Tsolis, 2008; Byndloss and Tsolis, 2016). T4SS also contributes to granulomatous inflammation in the spleen, a typical histopathological lesion of *Brucella* infection in mice (Byndloss and Tsolis, 2016). The secretion systems and secretomes of *Brucella* were recently computationally analyzed, resulting in the prediction of 36 host-pathogen interactions between sheep (*Ovis aries*) and *B. melitensis* proteins (Sankarasubramanian et al., 2016).

Unlike other type IV systems which are expressed extracellularly, transcription of the *virB* operon is expressed intracellularly within macrophages and phagosome acidification plays an important role in intracellular signal inducing *virB* expression (Boschiroli et al., 2002).

BvrR/BvrS Two-Component Regulator System

An important virulence mechanism of *Brucella* is the BvrR/BvrS two component regulatory system. This regulatory system acts as a signaling pathway which is necessary for modulation of the host cell cytoskeleton and controls the carbon and nitrogen metabolic functions (Sola-Landa et al., 1998; Martínez-Núñez et al., 2010; Viadas et al., 2010; Rossetti et al., 2017). The BvrR/BvrS regulon also includes the expression of additional transcriptional regulators among 127 differentially regulated genes (Viadas et al., 2010). Among the genes regulated by BvrR/BvrS, there are 10 transcriptional regulators, which were found the *vjbR* that participate in the regulation of the expression of the *VirB* locus that encodes the T4SS necessary to *Brucella* intracellular survival (Comerci et al., 2001; Delrue et al., 2004; de Figueiredo et al., 2015). Deletion of *Brucella vjbR*, a LuxR-like transcriptional regulator, greatly attenuates intracellular survival of *B. melitensis* (Weeks et al., 2010).

The BvrR/BvrS system also regulates transcription of at least two major outer membrane proteins (OMPs), such as *Omp22* (*Omp3b*) and *Omp25a* (*Omp3a*) but these proteins are not decisive for virulence (Manterola et al., 2007). The two components of this system are BvrR, a cytoplasmic regulator protein, and BvrS, a sensor protein with histidine-kinase activity located in the cell membrane (López-Goñi et al., 2002; Martínez-Núñez et al., 2010). In the absence of a functioning BvrR/BvrS, brucellae is unable to replicate intracellularly and is avirulent in the mouse model (Sola-Landa et al., 1998).

Lipopolysaccharide (LPS) O-Antigen

The bacterial cell envelope is the major point of interaction between brucellae and the host and as such, molecules within the bacterial cell envelope play a significant part in the infection process as brucellae escaping both innate and adaptive immunity and enabling the bacterium to reach its intracellular niche (Haag et al., 2010). *Brucella* LPS is another virulence factor that contributes to initial survival of bacteria in macrophages and is required for resistance against both extra and intracellular antimicrobial mechanism of the host (Lapaque et al., 2005; Lapaque et al., 2006). *Brucella* LPS consists of three components: lipid A, the core oligosaccharide, and the O-antigen or O-side chain (Cardoso et al., 2006). However, *Brucella* LPS, unlike *Escherichia coli* LPS, is a weak inducer of innate immune system (Haag et al., 2010).

Characterization of the LPS phenotype of smooth or rough *Brucella* depends on the presence or absence of the surface exposed O-polysaccharide chain, respectively. Mutant rough strains are defective for survival in macrophages cultures, as well as *in vivo* in the mice model of brucellosis. These mutant strains are considerably less virulent (Young, 1995; Lapaque et al., 2005; Cardoso et al., 2006; Haag et al., 2010; Mancilla, 2015).

LPS prevents complement-mediated bacterial killing (Allen et al., 1998; Tumurkhuu et al., 2006), confers resistance against both the extra

and intracellular antimicrobial mechanism such as defensins lysozyme, lactoferrin nitric oxide and free radicals (Martínez de Tejada et al., 1995), and inhibits cell death (Pei and Ficht, 2004; Pei et al., 2006). Additionally, *Brucella* LPS masks recognition of the PAMPs (Pathogen-Associated Molecular Patterns) from immune-receptor recognition, and as a consequence, impedes, or attenuates pro-inflammatory responses and immune system activation (Forestier et al., 2000; Jiménez de Bagués et al., 2004). *Brucella* can block maturation of phagosome through the interaction of smooth LPS with lipid rafts on the surface of macrophages which contributes to the transient early fusion between *Brucella* phagosomes and lysosomes (Porte et al., 2003; Kim, 2015).

LPS is the major surface antigen and is recognized by the immune system by the Toll-like receptor 4 (TLR4)/MD2 complex (Bryant et al., 2010; Haag et al., 2010). LPS is the major antigen that is presented by the MHC II to B-cells (Forestier et al., 1999; Haag et al., 2010). However, *Brucella* LPS interacts with MHC II molecules in a way that prevents signaling and activation of MHC II dependent T-cells (Lapaque et al., 2006; Haag et al., 2010). The importance of a type 1 T helper (Th1) cell response against *Brucella* is supported by numerous research studies, particularly the roles of CD4⁺ and CD8⁺T cells (Oliveira et al., 2002; Skendros et al., 2007; Durward et al., 2012; Durward-Diioia et al., 2015).

Previous studies suggest that an antibody to LPS (O-polysaccharide) may contribute to protection, but the effectiveness of the T helper cell type 2 (Th2) humoral immune response remains unclear and the efficacy of rough *Brucella* vaccines contradicts the role of anti-LPS antibodies in protective immunity (Baldwin and Goenka, 2006).

Cyclic- β -1,2-Glucans

Brucella produces cyclic β -1,2-glucans, an additional virulence mechanism, which are important to survive intracellularly and to targeting them to their replicative niche in the endoplasmic reticulum within the host cell. *Brucella* needs to enter the host cell via lipid raft domains in order to

establish itself with success, and cyclic glucans plays a major role in the persistent infection in the host environment (Haag et al., 2010). Cyclic β -1,2-glucan is a molecule secreted into the periplasm of *Brucella* and is required for intracellular survival to avoid fusion of the phagosome with lysosomes (Arellano-Reynoso et al., 2005).

Cyclic β -1,2-glucan interferes with cellular trafficking and maturation of the *Brucella*-containing vacuole by disrupting cholesterol-rich lipid rafts present on phagosomal membranes and preventing the phagosome-lysosome fusion (Rossetti et al., 2017).

Internalization of smooth *Brucella* is facilitated in the macrophage cells by lipid raft attachment to the plasma membrane (von Bargen et al., 2012). Cyclic β -1-2 glucans of *Brucella* prevent phagosome maturation by interfering with lipid rafts, thus altering protein expression in the vacuolar membrane and excluding lysosomal proteins from the BCV (Arellano-Reynoso et al., 2005; Haag et al., 2010).

Lipid rafts can also be found intracellularly on phagosomes and have been proposed to be involved in phagosome maturation. Therefore, phagosomal lipid rafts present an ideal target for intracellular pathogens, which could influence intracellular signaling and/or trafficking by modifying phagosomal lipid raft domains (Arellano-Reynoso et al., 2005; Haag et al., 2010).

Challenges and New Trends

The understanding of *B. melitensis* pathogenicity requires the recent identification of the 3,000 genes involved which are essential for the survival of bacteria *in vivo* resulting in disease (Tan et al., 2015).

In the future, new genomics and omics technologies and software tools will be used to gain knowledge of the different mechanisms of pathogenesis of brucellosis. In fact, a deep understanding of *Brucella* virulence factors simultaneous regulation, the pathogen mechanisms, disease progression, and host immune response is crucial for the development of preventive and diagnostic methods against brucellosis.

Future perspectives include genomic and omic technologies and software tools to analyze and provide insights into the pathogenesis of brucellosis. The complete genome analysis of *Brucella* was the first step for gaining knowledge on different mechanisms and provides better genetic relationships between the *B. melitensis* species (Tan et al., 2015). This data provided a solid baseline for a better comprehension of infection of *Brucella*-host relations and in the future the information is fundamental for vaccine production and strategies to prevent and control of brucellosis in small ruminants (Salmon-Divon et al., 2018).

The next challenge is to understand how these 3,000 genes express and interact under different environments in the complex mechanism of pathogenesis of brucellosis.

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