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REVIEW

Dendritic cells and *Brucella* spp. interaction: the sentinel host and the stealthy pathogen

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Abstract

As dendritic cells (DCs) are among the first cells to encounter antigens, these cells trigger both innate and T cell responses, and are the most potent antigen-presenting cells. *Brucella* spp., which is an intracellular facultative and stealthy pathogen, is able to evade the bactericidal activities of professional phagocytes. Several studies have demonstrated that *Brucella* can survive and replicate intracellularly, thereby provoking impaired maturation of DCs. Therefore, the interaction between DCs and *Brucella* becomes an interesting model to study the immune response. In this review, we first will describe the most common techniques for DCs differentiation in vitro as well as general features of brucellosis. Then, the interaction of DCs and *Brucella*, including pathogen recognition, molecular mechanisms of bacterial pathogenesis, and intracellular trafficking of *Brucella* to subvert innate response, will be reviewed. Finally, we will debate diversity in immunological DC response and the controversial role of DC activation against *Brucella* infection.

Introduction

Dendritic cells (DCs) are professional antigen (Ag)-presenting cells (APCs) distributed throughout an animal's body that exhibit major histocompatibility complex (MHC) on their surface and are a good source of pro-inflammatory cytokines such as IL-12 (Janeway and Medzhitov 2002). DCs are involved in two main roles: Ag uptake and processing and linking innate and adaptive immunity. Infectious agents and inflammatory products can induce DC activation, upon which DCs migrate to regional lymphoid tissue, such as lymph nodes, spleen, and Peyer's patches (Banchereau and Steinman 1998; Pulendran et al. 2001).

In peripheral tissues, DCs are present as immature cells with a poor capacity to stimulate T cells but are highly equipped to capture Ag (Banchereau and Steinman 1998). When immature DCs capture microbial agents or their products by phagocytosis, they travel away from the infection site and translocate to the T cell areas of the proximal lymph nodes (Pulendran et al. 2001).

DCs interact with a variety of T cells and drive the immune response (Colonna et al. 2006). For instance, DCs expressing

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MHC-I interact with $CD8^+$ T cells and induce a cytotoxic immune response, while MHC-II^+ DCs interact with $CD4^+$ T cells and induce a mixed Th1/Th2 immune response (Itano et al. 2003; Mantegazza et al. 2013). $CD4^+$ T cells, in the presence of mature DCs and IL-12, become interferon-γ (IFNγ)-producing T cells. IFNγ activates microbicidal macrophage properties and promotes an inflammatory (Th1) response (Itano et al. 2003; Pulendran 2004). On the other hand, IL-4 produced by DCs induces $CD4^+$ T cells to differentiate into Th2 cells. Th2 cells secrete IL-4 and IL-5 and subsequently activate eosinophils as well as help B cells make Ag-specific antibodies (Hochrein et al. 2000).

Generation of DCs

DCs originate in bone marrow from a common precursor for macrophages and DCs, the macrophage and DC precursor (MDP). MDP gives rise to the common DC precursor (CDP), which serves as a common progenitor for conventional DCs (cDCs) and plasmacytoid DCs (pDCs) (Poltorak and Schraml 2015).

In vivo, the development of all DCs is mostly dependent on FMS-like tyrosine kinase 3 ligand (FLT3L). In bone marrow, FLT3L acts on MDP and/or CDP and activates different transcription factor cascades to give rise to different DC subsets in a steady state (Poltorak and Schraml 2015). Granulocyte-macrophage colony stimulating factor (GM-CSF) is the other important growth factor for DCs development. Although GM-CSF does not have a major role such as FLT3L in DC development, it seems to balance DC subsets. For example, GM-CSF decreases pDCs and $CD8\alpha^+$ DC differentiation by blocking interferon regulatory factor-8 (IRF8) via signal transducer and activator of transcription-5 (STAT5) (Zhan et al. 2012a).

GM-CSF and FLT3L have been used to generate DC subsets in vitro. Bone marrow cells stimulated with recombinant FLT3L give rise to three DC subsets (referred to as FL-DCs): pDCs and 2 DC equivalents to the cDC subset. Although FL-DCs do not express the same markers as their in vivo cDCs counterparts, they use the same transcription factors, produce similar cytokine and chemokine profiles, and have similar efficiencies for Ag presentation as splenic $CD8\alpha^+$ and/or $CD8\alpha^-$ DCs (Brasel et al. 2000; Naik et al. 2005).

Generally, GM-CSF is used in combination with IL-4 to generate immature DCs from peripheral $CD14^+$ monocytes or bone marrow cells, and a further maturation step with a cytokine cocktail (TNFα, IL-1β, and/or IL-6) or bacterial-Ag is required to maintain a DC phenotype (Soruri and Zwirner 2005). GM-CSF-derived DCs (referred as to GM-DCs) are equivalent to myeloid DCs ($CD11b^{high}$, $CD11c^+$, $33D11^+$, and $CD8\alpha^-$) and differentiation is independent of STAT3 (Zhan et al. 2012b).

GM-CSF plus IL-4 are broadly used for the generation of DCs; however, alternative methodologies such as the addition of inflammatory cytokines have been developed to obtain DCs. For example, IL-15 allows the generation of DCs equivalents to GM-DCs from $CD14^+$ monocytes without the use of GM-CSF (Saikh et al. 2001).

DCs subsets

Differences in surface marker molecules on DCs subsets might be indicative of differences in the nature of the T cell response (Pulendran et al. 1999). CD11c and MHC-II seem to be constitutively expressed in most DC subsets; however, these surface markers do not display all different phenotypes and functions of DC subsets. DC subsets are classified based on different phenotypic characteristics such as the expression of intracellular and surface markers, transcription factors used for their differentiation, and anatomic location (Schlitzer et al. 2015).

In the mouse, two well-characterized cDC subsets have been described: the cDC1 subset includes DCs expressing $CD8\alpha^+$ (in lymphoid organs) or CD103 (in peripheral organs), while the cDC2 subset is comprised of $CD4^+CD11b^+$ DCs in the spleen and $CD24^+CD11b^+$ DCs in nonlymphoid tissues such as the lung, intestine, and dermis. The cDC1 subset requires IRF8 and is the only group that expresses Toll-like receptor 3 (TLR3), whereas cDC2 subsets require transcription factors such as IRF4. Phenotypic and functional studies have revealed similarities between mouse and human cDCs; in humans, $CD141^+$ DCs and $CD1c^+$ DCs resemble mouse cDC1 and cDC2, respectively (Guilliams et al. 2014; Schlitzer et al. 2015).

pDCs are the other major subset of DCs that develop from the CDP in bone marrow. pDCs belong to a different lineage of DCs; pDCs express B220, CD303, CD304, and CD123 and produce large amounts of type-1 interferon (IFN-α) (IFN-producing cells or IPCs) (Seillet and Belz 2013).

In lymphoid and nonlymphoid tissues, different subsets of DCs have been found. For example, in the spleen, two separate subsets of DCs have been described: lymphoid DCs ($CD8\alpha^+$ $CD11b^-$ $CD205^+$ $CD24^+$; referred to as $CD8\alpha^+$) located at the T cell zone in the spleen and myeloid DCs ($CD8\alpha^-$ $CD11b^+$ $CD24^+$ $33D1^+$; referred to as $CD8\alpha^-$) in the red pulp at the marginal zone. These DCs subsets not only differ in their surface markers and location but also in their capacity to process Ag and cytokine production; (i.e., IL-12 production is restricted to lymphoid DCs) (Hey and O'Neill 2012).

DCs take up and process a great variety of Ags, including those derived from bacterial pathogens. Once DCs process bacterial Ags, they trigger different signaling pathways to induce the appropriate immune response. However, bacterial pathogens, especially those able to live in an intracellular niche, have developed a plethora of strategies to subvert DC responses. Here, we focused especially on the interaction between the bacterial pathogen *Brucella* spp. and DCs.

Brucella species are intracellular facultative Gram-negative pathogens that reside inside various host cell types, including

DCs. *Brucella* species avoid the host immune response by utilizing several clever strategies such as avoiding intracellular destruction mechanisms and inhibiting DC maturation, Ag presentation, and T cell activation (de Figueiredo et al. 2015). Thus, DCs could serve as a model to understand the *Brucella* pathogenesis and to identify new targets for vaccine development against brucellosis. In the following sections, we describe some important characteristics of the interaction between *Brucella* spp. and DCs.

The stealthy pathogen: *Brucella* spp.

Brucella species are facultative intracellular Gram-negative bacteria, noncapsulated and nonspore-forming. *Brucella* causes the zoonosis named brucellosis, also known as Malta fever. Brucellosis is endemic in many countries of the world, including Latin America, the Middle East, Africa, Central Asia, and the Mediterranean (Pappas et al. 2006).

To date, 11 *Brucella* species have been described and classified according to the preferred animal host: *B. melitensis* (sheep and goats), *B. abortus* (cattle), *B. suis* (swine), *B. canis* (dogs), *B. ovis* (sheep) and *B. neotomae* (woodrats), *B. ceti* (dolphins, porpoises and whales), and *B. pinnipedialis* (seals) (Pappas 2010). In the last few years, new species have been found in different sources: *B. microti* was found in voles and foxes, *B. inopinata* was isolated from a breast implant infection, and recently, *B. vulpis* was isolated from red foxes (Scholz et al. 2008, 2010, 2016).

Brucellosis: general features

Acute and chronic brucellosis

In both humans and animals, *Brucella* targets the mucosa mainly through the respiratory epithelium or conjunctiva, and then the bacterium is internalized by phagocytes leading to systemic dissemination. However, little is known about the specific cells that allow bacterial internalization.

In humans, *Brucella* infection is mainly acquired through the ingestion of unpasteurized dairy products containing the bacteria; unpasteurized cheese and raw milk are commonly involved in brucellosis outbreaks in underdeveloped countries. Brucellosis is associated with some occupational groups such as farmers, veterinarians, ranchers, and meat-packing employees who may have contact with sick animals (Seleem et al. 2010).

The clinical manifestations of human brucellosis comprise nonspecific symptoms; the most common include undulant fever, myalgia, arthralgia, chills, headache, and weakness. Approximately 10–30% of brucellosis patients will develop chronic infection and/or some complications such as spondylitis, arthritis, endocarditis, or neurobrucellosis (Dean et al. 2012; Guzmán-Hernández et al. 2016). In animals, *Brucella* causes spontaneous abortion, stillbirths, and decreased fertility and milk production in females, whereas males experience orchitis, epididymitis, and fertility issues (Seleem et al. 2010).

Brucella evades the innate immune response, impairing DC maturation and subverting macrophage and neutrophil killing; indeed, *Brucella* resists and survives the bactericidal actions of neutrophils but does not replicate inside these cells (Barquero-Calvo et al. 2007). At the initial stage of infection, *Brucella* induces an adaptive immune response involving the microbicidal activity of macrophages (activated by IFN-γ of $CD8^+$ and $CD4^+$ T cells), and then infected macrophages are eliminated by cytotoxic T cells, and *Brucella* opsonization/phagocytosis is induced by IgG antibodies (Ahmed et al. 2016). In the case of chronic brucellosis, $CD4^+$ and $CD25^+$ T cells increase in the spleens of infected mice, which exert an immunosuppressive state regulating T cell activity. In patients with chronic brucellosis, high levels of TGF-β have been found in their sera, and furthermore, no T cell proliferation after stimulation with *Brucella* antigens has been observed, indicating suppressed T cell function mediated by TGF-β (Elfaki and Al-Hokail 2009). The chronicity of brucellosis is due to *Brucella's* ability to survive inside professional phagocytic cells and to evade the host immune response. *Brucella* can infect different cell types, including epithelial cells, trophoblasts, B cells, DCs, macrophages, synoviocytes, and T cells (Baldwin and Goenka 2006; Giambartolomei et al. 2017; Goenka et al. 2012; Velásquez et al. 2012). *Brucella* persistence occurs in mononuclear phagocytic system tissues, but the bacteria can also be found in reproductive organs, bones, and joints (Ahmed et al. 2016). Once infection is established, *Brucella* uses infected cells as a replicative niche and reservoir, inhibiting apoptosis and promoting chronic infection (Baldwin and Goenka 2006). Omp31 from *B. melitensis* contributes to the apoptosis inhibition induced by TNFα in RAW264.7 macrophages by the classical and mitochondrial pathway (Zhang et al. 2016), while *B. abortus* induces the A20 protein in RAW264.7 macrophages and promotes apoptotic pathway inhibition (Wei et al. 2015).

In mice infected with *Brucella*, the spleen is the main organ in which the pathogen persists. Susceptible IL-12p40 KO (Knock out)-mice infected intranasally with *B. melitensis* showed spleen infection after 28 days postinoculation. The bacteria were located in T cells on the white pulp of the spleen, and the main cells harboring *Brucellae* were cells resembling M2a macrophages. These reservoir cells infected with *Brucella* expressed $CD11c^+$, $CD205^+$, and $Arg1^+$; these markers were observed also in lung cells infected with *Mycobacterium tuberculosis* (Hanot Mambres et al. 2015). The deficient Th1 response in KO mice favoring the differentiation of the M2a macrophages population is indicative of a

Th2 environment. Previously, Xavier et al. found that M2a macrophages were induced during chronic infection provoked by *B. abortus*; interestingly, macrophages containing high numbers of bacteria were observed (Xavier et al. 2013). Because M2a macrophage polarization is induced by IL-4/IL-13 via STAT6, bacterial colonization in the spleen and impairment of M2a macrophage surface markers in double KO mice (IL-12p40$^{-/-}$ STAT6$^{-/-}$) were expected. However, Hanot-Mambres et al. found that neither *Brucella* infection in IL-12p40$^{-/-}$ STAT6$^{-/-}$ mice nor the surface markers expressed in macrophages were affected. Based on the observations of Xavier et al. and Hanot-Mambres et al., it is probable that myeloid cells are the critical reservoir in the spleen that may promote chronicity during brucellosis.

Pathophysiology of brucellosis

There is controversy over the ability of *Brucella* to invade multiple sites of the gastrointestinal tract; for example, *B. abortus* has been isolated from the gastrointestinal tracts of calves previously infected by the oral route with spiked milk (Carpenter 1924). Additionally, Rossetti et al. inoculated *B. melitensis* (3×10^9 UFC) in the intestinal loops of calves, and while the bacteria were recovered after 15-min and 12-h postinoculation in the Peyer's patches, no histopathological damage was observed in the tissue (Rossetti et al. 2013). On the other hand, von Bargen et al. showed that mice infected with *B. melitensis* 16M (10^9 bacteria) by the oral route induced the formation of granulomas composed of epithelioid cells and a few neutrophils in the cervical lymph nodes (CLN), although no dissemination was observed (von Bargen et al. 2015).

At the onset of *Brucella* infection in mice (from 0 to 2 days postinfection), there are no morphological or cell distribution changes in the spleen; additionally, the bacteria may be detected in the liver at the sinusoids inside the Kuppfer macrophages. In the acute phase (from the third day to the second–third week postinfection) the number of bacteria increases in the organs, and cell infiltration is apparent. In the liver, granulomas are evident in the first week after infection; granulomas are composed of macrophages and DCs, accompanied by plasma cells and lymphocytes (de Figueiredo et al. 2015; Grilló et al. 2012; Yoneyama and Ichida 2005). The spleen in the acute phase, after the first week of infection, shows increased size with macrophage and neutrophil infiltration, while the bacterial burden remains constant (Enright et al. 1990). At the end of the acute phase, the numbers of macrophages, neutrophils, and B and T cells increase slightly, as well as levels of IL-12, IL-6, and IFNγ (Grilló et al. 2012; Murphy et al. 2001). In the chronic phase (ranging from 8 to 11 weeks postinfection), granuloma size in the liver increases, and multinucleated giant cells and polykarions are observed in the centers of the granulomas (Cheville et al. 1995). Similarly, granuloma number and size in the spleen increase. The initial chronic brucellosis phase starts around the second–third week, and bacterial burden slowly decreases at approximately 75 days postinfection. Moreover, splenomegaly decreases, and macrophage infiltration is reduced (Enright et al. 1990; Grilló et al. 2012); however, the liver shows clearance at approximately 3–4 weeks postinfection, whereas the spleen is highly colonized during the chronic stage (Grilló et al. 2012).

From outside to inside: how do DCs allow *Brucella* entry?

Once ingested, *Brucella* spread during transport through the digestive tract. Some research has focused on studying oral infection of *Brucella* in vivo. One such report by Rossetti et al. involved healthy calves infected with 10^9 CFU of *B. melitensis* in the distal jejunum and ileum (ligated ileal loop model). The authors demonstrated that *B. melitensis* modulates inflammatory responses, limiting intestinal histopathological lesions, invading Peyer's patches, and disseminating to the mesenteric lymph nodes to induce bacteremia (Rossetti et al. 2013). Likely, *Brucella* translocates through the mucosal epithelium and is taken up by the DC subset present in the subepithelial dome of the ileal Peyer's patch. This DC subset secretes high levels of lysozyme (termed LysoDCs) and is highly efficient at capturing *Salmonella enterica* serovar Typhimurium (hereafter referred as *S. typhimurium*) (Lelouard et al. 2010, 2012); however, further investigation is needed to clarify whether LysoDCs are involved in *Brucella* uptake.

In a retrospective study analyzing patients diagnosed with brucellosis acquired via the ingestion of unpasteurized contaminated dairy products, most bacteria were found in the cervical lymph nodes (CLNs) (43–83%) and occasionally disseminated to other organs. CLNs drain the oral cavity, and most of the bacteria acquired by the oral route reach this regional lymph node (von Bargen et al. 2015; Yamazaki et al. 2012). Upon arrival at the CLNs, bacteria are captured or disseminated. Therefore, CLNs represent an efficient trap and reservoir for pathogens able to cause chronic infections, and the tissues drained by CNLs are the most successful route of entry to the host for pathogens acquired orally. As mentioned above, ingestion of unpasteurized contaminated dairy products is the most common route by which people acquire *Brucella* infection; however, it has been demonstrated experimentally that this is not the most efficient route since the bacterial burden required to infect mouse ranges from 10^9 to 10^{11} CFU. In infections caused by pathogens transmitted via the oral route, regional lymphadenitis is more common than diarrhea, indicating that the upper mucosa is the normal site of entry for these pathogens instead of the gastrointestinal tract. Taking into consideration these observations, it has been proposed that *Brucella* is not a proper enteropathogen (Gorvel et al. 2009; von Bargen et al. 2015).

Another report described the oral infection of mice with *B. melitensis* 16M (10^9 bacteria/mouse) using spiked milk. Oral infection led to early colonization of the CLN, where *Brucella* proliferated and persisted up to 50 days postinfection (chronic steady state). Moreover, local inflammation with upregulation of IFNγ and monocyte/macrophage was observed, but DC infiltration was not (von Bargen et al. 2015).

Under steady-state condition, the airways and trachea contain four specific DC subsets: intraepithelial DCs ($CD11c^{high}$ $CD11b^{low}$ $CD103^+$), DCs localized at the submucosa ($CD11b^{high}$ $CD103^-$ $CD11c^{high}$), monocytic DCs ($CD11b^{high}$ $CD103^-$ MHC-II^+), and pDCs. Intranasal infection of mice with *S. typhimurium* increases the percentage of DCs located at the submucosa level, whereas intraepithelial DCs decrease. However, both of these DC subsets upregulate the expression of costimulatory molecules such as CD80 and CD86, indicating clear activation of the immune system in the lung. Since *Brucella* is considered highly infectious, the inhalation of *Brucella* aerosols from aborted placenta or through laboratory exposures also leads to infection. In the case of the intranasal route, mice inoculated intranasally with 5×10^5 *B. abortus* 2308 were compared with a group of mice infected with *S. typhimurium*. The results showed that pulmonary *B. abortus* infection did not change the distribution of pulmonary DC subsets in the lung nor upregulate the expression of costimulatory molecules from 24 h up to 5 days postinfection (onset to acute infection) (Archambaud et al. 2010; del Rio et al. 2010; Smither et al. 2009; Traxler et al. 2013; Vermaelen and Pauwels 2005).

Alveolar macrophages have a major role in protecting against *B. abortus* 2308 infection; macrophages inhibit DC action, and only when the macrophages are overwhelmed will DCs interact with the pathogen. Upon partial macrophage depletion, the induction of inflammatory response by DCs was observed (Archambaud et al. 2010). *Brucella* was able to survive and replicate inside macrophages and DCs; alveolar macrophages along with DCs transport *Brucella* to the mediastinal lymph node, contributing to the dissemination of infection from the lung to other parts of the infected host (de Figueiredo et al. 2015).

Another common route for *Brucella* infection in humans and animals linked to bacterial aerosols is the conjunctival route. Although the eye is considered an immune-privileged organ, a subset of immature and mature DCs were found in the human and mouse cornea, at the epithelium and stromal zone. At the normal cornea, cDCs and Langerhans DCs have been observed: $CD141^+$ (cDC1) and $CD1c^+$ (cDC2) in humans and $CD103^+$ (cDC1) and $CD11b^+$ (cDC2) in mice (Knickelbein et al. 2009, 2014). However, it is not clear which DC subset is involved in *Brucella* infection. Although the conjunctiva is a less common route of natural infection with *Brucella*, this route has been used for vaccination to control caprine and ovine brucellosis, employing a *B. melitensis* Rev. 1 live vaccine (Blasco 1997). The conjunctiva is related to the conjunctiva-associated lymphoid tissue (CALT); an interconnection in the mucosal system allowing a distal immune mucosal response when the local mucosa is stimulated has been demonstrated, and M cells might be involved in the translocation of antigens (Pulendran and Ahmed 2011). For example, when sheep were infected by the conjunctival route with 5×10^7 CFU of virulent *B. melitensis* H38, the animals developed acute systemic brucellosis located in the peripheral lymph nodes but eradicated the bacteria at the local site of inoculation (Suraud et al. 2008).

DCs-*Brucella* interaction

The first step in establishing an intracellular lifestyle is entry of the bacterium into host cell, followed by avoidance of any intracellular destruction mechanisms. Some *Brucella* surface molecules are involved in host cell entry. One of these molecules is the SP41 protein, found in *B. melitensis*, *B. abortus*, and *B. suis*; SP41 is encoded by the BMEI0216 locus from *B. melitensis* 16M, and its function is to mediate entry of the pathogen into epithelial cells (Castañeda-Roldán et al. 2006; Hernández-Castro et al. 2008). Moreover, the *efp* gene and the pathogenicity island Bab1_2009–2012 from *B. abortus* promote adhesion and internalization into macrophage and epithelial cells (Czibener and Ugalde 2012; Iannino et al. 2012).

Inside host cells, especially DCs, *Brucella* modulates the expression of surface molecules related to maturation, costimulation, migration and/or Ag presentation, as well as influences cytokine production (Billard et al. 2007a; Elfaki et al. 2015; Fabrik et al. 2013).

There is contradictory information in the literature regarding the effects of *Brucella* on DC maturation/activation. Therefore, we divided the following section into two parts: the first will show evidence of DC activation/maturation by infection/stimulation with *Brucellae* or their antigens (outer membrane proteins (OMPs), lipopolysaccharide (LPS) or heat-killed bacteria), and the second part will describe reports demonstrating impaired activation/maturation. Moreover, we include a table which summarizes all these findings (Table 1).

Evidence of DC activation/maturation

Zwerdling et al. (2008) observed the activation and maturation of GM-DCs infected with *B. abortus*. Infected GM-DCs upregulated CD80, CD83, CD80, CD40, CCR7, MCH-II, and MHC-I surface molecules and induced cytokines such as IL-6, IL-10, IL-12, and TNFα (required for the induction and modulation of adaptive immune response). GM-DC maturation was independent of bacterial viability since heat-killed *B. abortus* (HKBa) induced upregulation of costimulatory molecules,

Table 1 DCs subsets stimulated with *Brucella* and their antigens

Stimuli/dose	DCs subset	Time (stimulation/infection)	Results obtained	Reference
B. abortus 2308 (MOI = 300)	Human GM-DCs	24 h	↓CD80, CD83, CD86, IFNβ	Gorvel et al., 2014
B. suis 1330 (MOI = 5) *B. abortus* 2308 (MOI = 5)	Human GM-DCs	24 h	↓CD40, CD83, MHC-II, ↓CD86, TNFα, IL-12	Billard et al. 2007a, b
HKBa (*B. abortus* 2308) (MOI = 50) L-Omp19 (10–1000 ng/mL)	Human GM-DCs	24 h	↑CD80, CD86, CD83, CD40, CCR7, MHC-II ↑Il-12, IL6, IL-10, TNFα	Zwerdling et al. 2008
HKBa (*B. abortus* 2308) (10^9/mL)	Murine bmGM-Dcs	24 h	↑CD40, CD86, MHC-II ↑IL-12p40, TNFα	Macedo et al. 2008
B. abortus	Murine bm-GM-DCs	24 h	↓CD40, CD80, CD86, MHC-II ↓IL-12p70, IL-6, IFNβ, TNFα	Salcedo et al. 2008
B. abortus 2308 (MOI = 100)	Bovine moGM-DCs	24 h	↓CD80, CD86 ↓IL-12p40, IFNγ	Heller et al. 2012
B. abortus 2308 (MOI = 100)	Murine bmGM-DCs GM/FL-DCs FL-DCs GM/15-DCs	48 h 24 h	↑CD80, CD86, MHC-II ↑IL-10 ↑IL-12, IL-6, IFNγ, IFNβ, IL-1β ↑CCL2, CCR7	Papadopoulos et al. 2016
OMVs *B. melitensis* 16 M (40 μg/mL) OMVs *B. melitensis* VTRM1 (40 μg/mL)	Murine bmGM-DCs	12 h	↑IL-6, IL-4, IL-10 ↑IL-12, IFNγ, TNFα	Avila-Calderón et al. 2012
LPS from *B. abortus* 2308 Δ*wadC* (10 μg/mL)	Murine bmGM-DCs	24 h	↑CD86, MHC-II ↑IL-12, TNFα	Conde-Álvarez et al., 2012
LPS from *B. melitensis* 16 M Δ*wadC* (20 μg/mL) LPS from *B. melitensis* 16 M (30 μg/mL)	Human GM-DCs Murine FL-DCs Murine FL-DCs	24 h	↑TNFa, IL-12p40, IL-6, IL-10 CD80, CD40, CD86, MHC-II ↑IL-1β, IL-6, IL-12p70, IFNγ ↓CD80, CD40, MHC-II, CD86 ↓IL-10, IL-6, TNFα	Zhao et al., 2018
B. canis strain SCL (MOI = 200)	Human GM-DCs Canine GM-DCs	24 h	↑CD86, CD83 ↑IL-12p35, TNFα, IL-1β ↑CD86, MHC-II ↑IL-17A, IL-6, IL-12p35, IFNγ	Pujol et al. 2017
HKBa (*B. abortus* 2308) (10^8/mL)	Splenic CD11c$^+$ DCs (TLR9$^{-/-}$)	Overnight	↓IL-12	Huang et al. 2005
HKBa (*B. abortus* 119.3) (10^8/mL)	Splenic CD11c$^+$ DCs Splenic CD11c$^+$ DCs (TLR2$^{-/-}$)	15 h	↑IL-12 ↓IL-12	Zhang et al. 2012
B. abortus RB51 (MOI = 10) *B. abortus* 2308 (MOI = 100)	Murine bmGM-DCs	24 h	↑MHC-II, CD40, CD86 ↑IL-12, TNFα ↓MHC-II, CD40, CD86 ↓IL-12, TNFα	Surendran et al. 2012
B. abortus 2308 RNA (2 μg/mL)	Murine bmGM-DCs	24 h	↑IL-6, IL-12, TNFα	Campos et al. 2017
B. abortus 2308 (MOI = 100)	Murine bmGM-DCs (IRAK-4$^{-/-}$)	24 h	↓IL-12, TNFα	Oliveira et al. 2011

↓Downregulation

↑Upregulation

chemokines receptors, and cytokines. GM-DCs maturation was attributed to *Brucella* lipoproteins; GM-DCs stimulated with lipidated Omp19 induced the upregulation of surface molecules and cytokines, whereas purified *B. abortus* LPS or unlipidated Omp19 did not (Zwerdling et al. 2008).

Using murine bone marrow–derived DCs (differentiated with GM-CSF plus IL-4; referred as bmGM-DCs), Macedo et al. observed the upregulation of costimulatory molecules (CD40 and CD86), MHC-II, as well as significant cytokine production (TNFα and IL-12p40) at 24-h poststimulation with HKBa relative to unstimulated cells (Macedo et al. 2008).

B. melitensis, *B. abortus*, and *B. suis* are the main species causing infection in humans and are mainly transmitted zoonotically (Guzmán-Hernández et al. 2016). A large number of studies have focused on these *Brucella* species and their interactions with the host due to the importance, incidence/frequency, and virulence of infection. Compared with the three species mentioned above, *B. canis* and *B. ovis* are the only two natural rough *Brucella* species that infect their animal host. *B. canis*, with a lower incidence of human cases, also represents a public health risk; however, until now, no studies examining its interaction with DCs have been reported (Marzetti et al. 2013). In humans, *B. canis* causes a mild or asymptomatic infection, whereas in dogs, like other animals, brucellosis induces abortion in females and epididymitis/orchitis in males, as well as some complications such as diskospondylitis (Wanke 2004). It is possible that differences in human and canine brucellosis may be attributed to differences in the immune response. When infected with *B. canis*, human and canine GM-DCs upregulate the expression of CD86, CD83, and class II molecules 24-h postinfection. On the other hand, cytokine gene profile expression differs: while canine GM-DCs overexpress IL-17A, IL-6, IFNγ, IL-12p35, human GM-DCs overexpress IL-12p35, TNFα, and IL-1β. At the protein level, the cytokine profiles are very similar; high production of INFγ and IL-17A in canine GM-DCs and IL-12 and TNFα in human GM-DCs. Clearly, *B. canis* elicits a Th1 response in human GM-DCs that is essential for the pathogen eradication, and this is likely the reason symptoms in humans are less severe. GM-DCs infected with *B. canis*, elicit a Th1/Th17 cytokine profile (Pujol et al. 2017). The role of the Th17 response in brucellosis is not clear yet; IL-17 has been implicated in osteoarticular complications in brucellosis. Additionally, Th17 cells producing IFNγ/IL-17 are related to inflammatory disorders (Giambartolomei et al. 2017). Another intracellular pathogen, *M. tuberculosis* induces a combined Th1/Th17 response and the formation of granulomas (Bystrom et al. 2015; Lyadova and Panteleev 2015). It is likely that the IFNγ/IL-17 cytokine profile elicited by *B. canis* in canine DCs is associated with osteoarticular complications in dogs such as diskospondylitis.

Evidence of impaired DC activation/maturation

Despite the stealthy ability of *Brucella* to reside inside professional phagocytes and impair the immune response, the role of *Brucella* in DCs activation/maturation seems to be contradictory. Human monocyte-derived GM-DCs infected with smooth *B. suis* or *B. abortus* decreased the expression of maturation surface markers (chemokine receptor CCR7, CD83), costimulatory molecules (CD86, CD40), and MHC-II relative to GM-DCs infected with rough mutants. Moreover, *Brucella* infection decreased Ag presentation, as well as the production of the inflammatory cytokines IL-12 and TNFα (Billard et al. 2007b). TNFα is essential for DC maturation; in a previous report, *B. suis* Omp25 impaired TNFα production on THP-1 monocytes (Jubier-Maurin et al. 2001). Recently, it was demonstrated that *B. suis* Omp25 dysregulates microRNA expression on porcine alveolar and murine macrophages, leading to the inhibition of NF-κB signaling and TNFα suppression (Billard et al. 2007b; Luo et al. 2018). The decrease in inflammatory cytokines and the poor T cell stimulation of *Brucella*-infected GM-DCs avert the induction of a Th1 immune response, which is essential for eradicating intracellular bacteria (Billard et al. 2007b).

Salcedo et al. (2008) observed decreased expression of CD40, CD80, CD86, MHC-II, and low cytokine concentrations of IL-12p70, TNFα, IL-6, and IFN-β in bmGM-DCs infected with live *B. abortus* relative to bmGM-DCs infected with *S. typhimurium*. These results suggest DC maturation impairment in cells infected with *Brucella* (Salcedo et al. 2008).

In a comparative study, human monocyte-derived GM-DCs were infected with different intracellular bacterial pathogens: *Orientia tsutsugamushi* the etiologic agent of scrub typhus, *Coxiella burnetii* responsible for Q fever, and *Brucella*. Although these bacterial pathogens have an intracellular lifestyle, they have different preferential niches for their replication; while *B. abortus* 2308 and *C. burnetii* reside in an intracellular vacuole, *O. tsutsugamuchi* is located in the cytoplasm (Benoit et al. 2008; Gorvel et al. 2014; Tantibhedhyangkul et al. 2013). Gorvel et al. (2014) infected GM-DCs with *B. abortus* 2308, *C. burnetii*, and *O. tsutsugamuchi* for 24 h. Compared with GM-DCs infected with *C. burnettii* or *O. tsutsugamushi*, *B. abortus*-infected GM-DCs showed impaired immune responses. However, the expression of CD83 (a DC maturation marker), and costimulatory molecules (CD80 and CD86) was relatively higher in *O. tsutsugamushi*-infected GM-DCs, than *B. abortus* 2308- and *C. burnetii*-infected cells (Gorvel et al. 2014). Despite the intracellular lifestyle of these pathogens, notable differences in GM-DC immune responses were observed; further analysis involving transcriptomics was performed on infected GM-DCs. Transcriptomic analysis revealed high levels of IFN-β in GM-DCs infected with *O. tsutsugamushi*, whereas IFN-β expression was impaired in

Brucella- and *C. burnetti*-infected GM-DCs. According to the results obtained, IFN type I was impaired due to defective activation of p38 in GM-DCs infected with *B. abortus* 2308 and *C. burnetii* (Gorvel et al. 2014).

Compared with human or murine DCs, bovine monocyte-derived GM-DCs are not permissive for *B. abortus* proliferation. Heller et al. (2012) found low expression of costimulatory molecules (CD80 and CD86) and cytokines (IL12p40 and IFNγ) in bovine monocyte-derived GM-DCs infected with *B. abortus*, but no bacterial proliferation was observed (Heller et al. 2012). While the proliferation of bacteria in macrophages is commonly observed, intracellular bacterial proliferation in DCs is poor, probably because phagolysosome acidification is decreased in these cells. It is well known that *Brucella* requires phagolysosome acidification for the expression of crucial virulence factors for intracellular survival (Starr et al. 2008).

Does *Brucella* truly impair DC activation/maturation?

It is evident that there are discrepancies between the results reported in the literature regarding DC activation/maturation, probably due to the DC subsets used in experiments, the species of *Brucella* or antigens, and the time points used for stimulation/infection, among others.

Billard et al. (2007b) found low activation levels of CD83, costimulatory molecules, and chemokine receptors at 48-h postinfection in human GM-DCs infected with *B. suis*, whereas Zwerdling et al. (2008) observed high expression levels of surface molecules at early time points (24-h postinfection) in human GM-DCs infected with *B. abortus*. Likely, the induction of surface molecules reaches its maximum intensity at different time points after infection, explaining why differences were observed between these reports. Moreover, Billard et al. (2007b) performed GM-DC differentiation for 5 days, while Zwerdling et al. (2008) did so for 6 days. Additionally, Zwerdling et al. (2008) induced a further maturation step using different antigens (*Escherichia coli* LPS, *B. abortus* LPS, HKBa, Pam3Cys, Lipidated-Omp19, and Unlipidated-Omp19), whereas Billar et al. stimulated the cells after differentiation with cytokines. Despite the impaired GM-DC maturation observed in the experiments of Billard et al., *Brucella* infection was slightly detected based on the immune response, since low cytokine levels were induced. Based on the observation of *Brucella* infection in DCs, it can be hypothesized that DC activation mediated by *Brucella* is present at the onset of the immune response, while at later time points, the pathogen might avert a Th1 response by establishing chronic infection through different host immune system evasion mechanisms (Billard et al. 2007a, 2007b; Zwerdling et al. 2008).

Reports of murine DCs infected with *Brucella* have presented contrasting information. Macedo et al. (2008) used heat-killed *B. abortus* 2308 (1000 bacteria/cell) to infect murine DCs, observing upregulation of costimulatory molecules, as well as significant cytokine production. Salcedo and colleagues used living *B. abortus* 2308 strain (20–50 bacteria/cell) and observed decreased expression of surface molecules and low cytokine concentrations.

The stealthy nature of *Brucella* hampers the immune response of DC subsets

Human and mouse GM-DCs have been the most commonly used model to study *Brucella*-DC interaction. Since *Brucella* can disseminate and reach different lymphoid tissues such as the spleen, the bacteria can interact with different DC subsets.

Papadopoulos et al. (2016) demonstrated that *Brucella* can infect different bone marrow-derived DC subsets. Bone marrow cells were differentiated in GM-DCs, FL-DCs (pDCs, and $CD8\alpha^+$, and $CD11b^+$ equivalent to cDCs), GM/FL-DCs (GM-CSF plus FLT3L DCs), and GM/15-DCs (GM-CFS plus IL-15 DCs) and then infected with virulent *B. abortus* 2308 strain. In the case of GM/FL-DCs, GM-CSF decreased $CD8\alpha^+$ DCs and pDCs, while $CD11b^+$ DCs increased these subsets. On the other hand, GM/15-DCs produced higher levels of IFNγ and IL-12 (promoting Th1 response) than GM-DCs (Brasel et al. 2000; Naik et al. 2005; Papadopoulos et al. 2016). When DC subsets were infected with *Brucella*, different infection kinetics were observed; for example, FL-DCs and GM/15-DCs showed higher infection levels at early time points than GM/FL-DC or GM-DCs. Brasel et al. (2000) observed the similar efficiency of FL-DCs and GM-DCs for ovalbumin (OVA) uptake; however, *Brucella* uptake may be mediated by different mechanisms (i.e., lipid rafts and/or phagocytosis). At late times postinfection, *Brucella* uptake levels were similar in all different DC subsets. On the other hand, GM-DCs and GM/FL-DCs showed late maturation (high levels of CD86, CD80, and MHC-II at 48 h), while GM/15-DCs and FL-DCs (only $CD8\alpha^+$ and $CD11b^+$ DCs) showed early maturation at 24-h postinfection. GM-DCs and GM/FL-DCs induced high levels of IL-10 corresponding to late maturation of cells. FL-DCs and GM/15-DCs showed higher expression of INFγ, IL-12, IL-6, IFNβ, and IL-1β as well as higher levels of chemokine and chemokine receptor (CCL2 and CCR7) corresponding to early maturation. Moreover, in DC subsets with inflammatory cytokine profiles, *Brucella* replication was lower than that in GM-DCs and GM/FL-DCs (Papadopoulos et al. 2016).

During *Brucella* infection, IL-10 production is commonly observed; mouse GM-DCs produce IL-10 at 24-h postinfection and splenocytes from infected mice produced high levels of this cytokine up to 6 weeks postinfection. In this sense, DCs, $CD4^+$ T cells, and macrophages from the spleen are the main IL-10

producers (Corsetti et al. 2013). IL-10 is considered an immunoregulatory cytokine that is able to suppress IL-12 production and modulate an excessive Th1 response (Couper et al. 2008). During *Brucella* infection, IL-10 production at early times is crucial for evolution to chronic brucellosis, which includes the inhibition of pro-inflammatory cytokines. Xavier et al. (2013) found that at early times during *Brucella* infection, IL-10 produced by $CD4^+CD25^+$ T cells has a regulatory effect during the acute phase, which decreases inflammation and tissue damage; this immune response protects bacteria from being eliminated. Valuable data were obtained in experiments using IL-10 KO mice infected with *B. abortus*. IL-10 KO mice showed lower bacterial loads than wild-type mice. Additionally, GM-DCs from IL-10 KO mice produced higher levels of pro-inflammatory cytokines than GM-DCs from wild-type mice. Altogether, these results indicate that IL-10 has a detrimental effect on the protective immune response against *Brucella* (Couper et al. 2008); Xavier et al. 2013).

The stealthy nature of *Brucella* is attributed mainly to the smooth LPS on its surface. Typical LPS is composed of lipid A, a core oligosaccharide, and an O-side chain polysaccharide. *Brucella* LPS is an unbranched homopolymer ranging from 96 to 100 glycosyl subunits; the O-side chain is linked to a core oligosaccharide, and the lipid A is linked to the core oligosaccharide (Cardoso et al. 2006). LPS plays an important role in *Brucella* pathogenesis, protecting from cellular cationic peptides, reactive oxygen species, and complement-mediated lysis and is involved in invasion. *Brucella* LPS is less cytotoxic than enterobacterial LPS (Cardoso et al. 2006; von Bargen et al. 2012). There is some controversy as to whether or not *Brucella* LPS hampers the DC immune response; as mentioned above, some authors have reported DC maturation/activation inhibition by smooth *Brucella* strains, whereas DCs infected with rough strains showed maturation/activation and proinflammatory cytokine profiles.

Outer membrane vesicles (OMVs) are nanovesicles released from the outer membrane of Gram-negative bacteria. OMVs contain proteins from the outer membrane, periplasm, and cytoplasm, as well as LPS. These vesicles have been reported in *B. melitensis* and *B. abortus*. Purified vesicles from *B. melitensis* 16M and the rough mutant *B. melitensis* VTRM1 were used to measure the levels of cytokine expression in murine bmGM-DCs at different time points. The results revealed early expression of IL-12, IFNγ, and TNFα in bmGM-DCs stimulated with OMVs from *B. melitensis* rough strain but not expression with OMVs from a smooth strain (Avila-Calderón et al. 2012). These differences could be attributed to the O-side chain lacking the LPS of OMVs from the rough strain.

Conde-Álvarez et al. (2012) found a relationship between the core oligosaccharide and immune response impairment in DCs. In their experiments, the authors used the *B. abortus wadC* mutant; the *wadC* gene encodes a mannosyltransferase that is important for the assembly of complete LPS. The *B. abortus wadC* mutant exhibited a partially defective core oligosaccharide but an intact lipid A and O-side chain. bmGM-DCs were stimulated with LPS purified from the *wadC* mutant and wild-type strain. The mutant LPS induced higher production of IL-12, TNFα, and CD86 and MHC-II overexpression, but cells stimulated with the wild-type LPS did not (Conde-Álvarez et al. 2012). Similar experiments were performed by Zhao et al. (2018) using human monocyte-derived GM-DCs stimulated with *B. melitensis-wadC* mutant LPS and wild type LPS, the production of IL-12p70, IL-6, and TNFα was observed only in cells stimulated with mutant LPS (Zhao et al. 2018).

Clearly, immune response impairment in GM-DCs is due to the core oligosaccharide in *Brucella* LPS, but this is not the rule in all DCs subsets. For example, in the case of FL-DCs stimulated with *B. melitensis* wild-type LPS, the $CD11b^+$ and $CD8\alpha^+$, but not pDC, subsets upregulated the expression of CD86, CD80, CD40, and MHC-II molecules and secrete significant amounts of TNFα, IL-12p40, IL-6, and IL-10. Additionally, upregulation of surface molecules and cytokine production was observed in FL-DCs DCs stimulated with *B. melitensis-wadC* mutant LPS but was higher than that observed in FL-DCs stimulated with wild-type LPS. However, despite the activation profiles elicited in $CD11b^+$ and $CD8\alpha^+$ cells stimulated with wild-type LPS, these DCs were not able to induce the proliferation of $CD8^+$ or $CD4^+$ T cells (Zhao et al. 2018).

It has been reported that human or mouse GM-DCs treated with *B. abortus* or pure wild-type LPS hampered pro-inflammatory cytokine profiles and demonstrated an inability to upregulate costimulatory molecules (Conde-Álvarez et al. 2012; Papadopoulos et al. 2016). Papadopoulos et al. observed maturation at 48-h postinfection and high production of IL-10 in murine GM-DCs infected with *B. abortus* 2308. Conde-Álvarez et al. (2012) did not observe costimulatory molecule expression in murine GM-DCs after 72 h of stimulation with *B. abortus* wild-type LPS. In addition, in both studies, upregulation of surface molecules and induction of a Th1 cytokine profile in $CD11b^+$ and $CD8\alpha^+$ DC subsets (FL-DCs) was observed. Papadopoulos et al. observed IFNγ secretion at 24-h postinfection and late upregulation of IL-10 production (48 h) when DCs were infected with whole *B. abortus* 2308. Late IL-10 induction potentially counteracts IFNγ effects and impairs DC response (Conde-Álvarez et al. 2012; Papadopoulos et al. 2016).

Molecular interaction: how does *Brucella* avoid DCs immune response?

Brucella spp., are recognized by TLRs from DC subsets and activate intracellular pathways for cytokine production

TLRs are involved in recognizing pathogen-associated molecular patterns (PAMPs), and in some cases, DC activation is

initiated by these receptors. TLRs have an extracellular leucine-rich repeat (LRR) domain, and the intracytoplasmic domain Toll/IL-1 receptor domain (TIR domain) homologous to the IL-1 receptor family (IL-1R) is crucial for intracellular signaling. Myeloid differentiation factor 88 (MyD88) is an adaptor protein which possesses a TIR domain and associates with IL-1R family members including TLRs (Kaisho and Akira 2001).

Based on their observations, Zwerdling et al. (2008) proposed that *Brucella* signals through TLR2, since GM-DCs stimulated with pure *Brucella* Omp19 impaired cytokine production when this receptor was blocked. On the other hand, Huang et al. (2005) observed a prominent role for TLR9 and MyD88 in $CD11c^+$ DCs isolated from the spleen for IL-12 production after the cells were treated with HKBa (Huang et al. 2005; Zwerdling et al. 2008). TLR2 and TLR4 are superficial receptors at the cytoplasmic membrane and are recruited at the site of the pathogen interaction during phagocytosis, whereas TLR9 is an intracellular receptor that recognizes distinct patterns of nucleic acids in endosomes (Kaisho and Akira 2001).

Zhang et al. (2012) observed that TLR2, but not TLR4 is required for HKBa phagocytosis in $CD8\alpha^-$ and $CD8\alpha^+$ DCs isolated from mouse spleen. HKBa phagocytosis induced the production of TNFα and IL-12; TLR2 was important for TNFα production, while TLR9 was related to IL-12 production and was MyD88 signaling-dependent. However, TLR4 was not required for the production of any cytokines in both DC spleen subsets. Additionally, it was demonstrated that TLR2-TLR9 cooperation was necessary for the production of pro-inflammatory cytokines. HKBa interacts with TLR2 in splenic DCs and signals through p38, leading to phagocytosis, and signals for TNFα production, resulting in phagolysosome fusion; then TLR9 interacts with bacterial DNA in late endosomes, signaling for IL-12 production (Zhang et al. 2012). On the other hand, in bmGM-DCs infected with *B. abortus*, TLR6 expression was higher than TLR2 expression, and TLR6 was found to be more important for IL-12 and TNFα production (de Almeida et al. 2013).

To identify the differential roles of TLRs in *Brucella* elimination in a pulmonary infection model, bmGM-DCs from TLR2, TLR4, and TLR9 KO mice were infected with rough vaccine *B. abortus* RB51 and smooth virulent *B. abortus* 2308. bmGM-DCs infected with strain RB51 showed higher levels of MHC-II, CD40, and CD86 expression mediated by TLR2, 4, and 9, as well as IL-12 production mediated by TLR2 and TLR4, than the smooth virulent strain. GM-DCs from wild-type mice showed impaired activation. DC activation was induced using MOI = 100 in the case of *B. abortus* 2308, while MOI = 10 was necessary in the case of the rough strain to activate cells (Surendran et al. 2012).

Clearly, IL-12 production is necessary for an appropriate Th1 response against brucellosis. TLR4 is the only receptor that signals through both TRIF (TIR-domain-containing adapter-inducing interferon-β) and MyD88 upon stimulation and acts synergistically in DCs for IL-12 production (Krummen et al. 2010). In this sense, Zhang et al. (2012) demonstrated IL-12 production-independent TLR4 signaling by splenic DCs infected with *B. abortus*; however, Surendran et al. (2012) proposed TLR4 participation in IL-12 production during *B. abortus* infection.

Recently, TLR3 and TLR7 have been implicated in sensing *Brucella* RNA. *B. abortus* RNA-induced IL-6, IL-12, and TNFα production in murine bmGC-DCs; this cytokine production was TLR-dependent and occurred via MAPK/NF-κB signaling. Although TLRs were required to sense bacterial RNA and cytokine production, they were not necessary for *Brucella* eradication in vivo (Campos et al. 2017).

Almost all TLRs signal through MyD88, but alternative downstream signaling pathways could be involved in cytokine production. For example, Zhang et al. (2012) observed TNFα production mediated by TLR2 via p38 activation. On the other hand, TLR2 also activates ERK1/2 and exerts negative feedback for IL-12 production mediated by TLR9.

Upon activation, TLRs signal through the common adaptor molecule MyD88 (except TLR3) and recruit interleukin-1 (IL-1) receptor-associated kinase (IRAK) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6). Once TLRs interact with MyD88, IRAK is activated by TAK1 (transforming growth factor-activated kinase) in a phosphorylation-dependent manner. Activated IRAK associates with TRAF6, leading to the activation of JNK (Janus kinase) and culminating in IκB degradation and NF-κB translocation to the nucleus for the transcription of pro-inflammatory cytokine genes such as IL-12 and TNFα (Kawai and Akira 2007; Takeda and Akira 2015; Underhill and Ozinsky 2002).

Additionally, IRAK-4 was found to be involved in the production of IL-12 and TNFα in macrophages and DCs infected by *Brucella*. Bone marrow–derived macrophages and bmGM-DCs from $IRAK\text{-}4^{-/-}$ mice were stimulated with live *B. abortus* 2308, HKBa, TLR2, and TLR9 agonists, IRAK-4 deficient macrophages and bmGM-DCs showed impaired production of TNFα and IL-12, demonstrating an essential role for IRAK-4 in the production of pro-inflammatory cytokines upon *Brucella* recognition by TLRs (Oliveira et al. 2011).

Brucella TIR proteins and other virulence factors act as immune modulators of DCs

A more detailed study of the interaction of mouse GM-DCs and *B. abortus* 2308 was performed using microarrays. Functional expression analysis showed that not only type I IFN response but also MAPK p38 activation were impaired (Gorvel et al. 2014). It has been reported that the O-side chains of LPS from *B. melitensis* 16M, *B. abortus* 2308, and *B. suis* 1330 are able

to restrict the activation of p38 in murine J774A.1 macrophages. Additionally, the production of inflammatory cytokines and chemokines depends on p38 MAPKs (Jiménez de Bagüés et al. 2005). Taking into account these data, we can show that *Brucella* modulates DC maturation by impairing the type I IFN and the immune response, allowing bacterial intracellular persistence and chronic infection.

Another strategy used by *Brucella* to avoid the host immune response involves blocking or impairing TLRs signaling pathway through bacterial TIR homologs (Rana et al. 2013). Cirl et al. identified bacterial TIR-domain proteins in uropathogenic *E. coli* CFT073 and *Brucella* species, referred as TIR-domain-containing proteins C (TcpC) and B (TcpB, also known as BtpA), respectively (Cirl et al. 2008). The *btpA* gene was found in *B. melitensis* and *B. abortus*, but not in the *B. suis* genome; the genetic evidence proposes that these genes were acquired recently via a phage-mediated integration event. The BtpA protein interferes with NF-κB activation mediated by TLR4 and TLR2 signaling, as well as cytokine production and DC maturation (Radhakrishnan et al. 2009).

For TLRs, activation adaptor proteins are recruited, one of which is Toll-interleukin-1 receptor domain-containing adaptor protein (TIRAP, also known as MAL; MyD88 adaptor protein). TIRAP is an adaptor molecule for the MyD88-dependent pathway derived from TLR2 and TLR4-signaling (Kawai and Akira 2007; Underhill and Ozinsky 2002). Radhakrishnan et al. (2009) observed no interaction between BtpA, TLR2, TLR4, or TIRAP to inhibit NF-kB activation in HEK293 cells transfected with the *btpA* gene. Interestingly, BtpA was found to mimic TIRAP function during TLRs signaling; BtpA likely competes with MyD88 and blocks downstream signaling (Radhakrishnan et al. 2009). On the other hand, recombinant BtpA was able to induce the degradation of TIRAP, while other TLR components were not affected. It has been suggested that the interaction of BtpA and TIRAP facilitates the ubiquitination of TIRAP for its degradation (Fig. 1) (Sengupta et al. 2010).

MyD88 possesses an amino-terminal death domain (DD) involved in cell death and signaling. Through the interaction of their DDs, MyD88 recruits the IRAK-1 and IRAK-4 kinases (Loiarro et al. 2009). According to a fragment complementation assay, it was possible to determine that BtpA interacts directly with MyD88 and TIRAP through the DDs. The interaction between BtpA and MyD88 was stronger than that observed with BtpA-TIRAP. Although BtpA interacts strongly with MyD88, the interaction does not impair downstream signaling (Fig. 1) (Chaudhary et al. 2012).

A second TIR-domain-containing protein (BAB1_0756) in the *Brucella* genome was discovered and designated as BtpB. BtpB is a potent inhibitor of TLR2, TLR4, TLR5 and TLR9 signaling. Infection of murine bmGM-DCs with *B. abortus* BtpB mutant led to the reduction of TNFα and IL-12. Some differences were observed between BtpA and BtpB; for example, BtpA, but not BtpB impaired TNFα production, while BtpB decreased the expression of MHC-II, CD40, CD86, and CD80 in bmGM-DC cells. However, both proteins contributed to the control of DC activation during *Brucella* infection (Salcedo et al. 2013). Based on this evidence, BtpA may affect TIRAP functions but could also interact directly with MyD88 in a DD-domain dependent manner, although not with the TIR domain. BtpB also showed stronger MyD88-binding and likely blocks TLRs that are dependent on MyD88 signaling (Fig. 1) (Salcedo et al. 2013).

Concluding remarks

Brucella invades and replicates in a variety of host cells. A high percentage of the studies to examine *Brucella* pathogenesis, host-pathogen interaction, virulence, etc., are performed in macrophages, and in some cases, these cells are the preferential niche for intracellular replication. However, there is no doubt that DCs are an intracellular niche for *Brucella* species, and their migratory properties allow bacterial dissemination. With the exception of bovine-derived DCs, this pathogen infects and replicates inside DCs. *Brucella* recognition at the outside of the eukaryotic cell is mediated by TLRs and signaling triggers the immune response, as shown by cytokine production. Despite discrepancies regarding DC activation/maturation after *Brucella* infection, it is clear that *Brucella* infection is detected by murine or human GM-DCs since the bacteria drive low pro-inflammatory cytokine levels. Unlike other intracellular pathogens such as *Salmonella*, which induces high levels of pro-inflammatory cytokines and the expression of activation/maturation surface molecules, *Brucella* is able to subvert or "delay" the DC response, impairing the inflammatory response or the expression of surface molecules. At later times postinfection, *Brucella* exerts immunomodulatory mechanisms to avoid a protective immune response, promoting intracellular trafficking and reaching its intracellular niche by blocking TLR signaling and cytokine production.

It is evident that there is a controversy regarding whether infection with *Brucella* prevents or promotes the activation/maturation of DCs. In part, this contradiction is due to a large number of variables that are managed in experiments using DCs, for example, the bacterial strains used, the bacterial dose and the time for infection/stimulation. It is impossible to homogenize all methodologies, since one DC subset cannot be used because, as we have emphasized in this review, there are several DC phenotypes. On the other hand, the genetic variation of *Brucella* strains, even though the same bacterial species are used, including references or clinical isolated strains, may affect the results. Similarly, it is impossible to control the physiological and genetic variations of the animals and donors used to obtain DCs. Undeniably, the nature of the stimulus used causes variations in the results, since stimulation

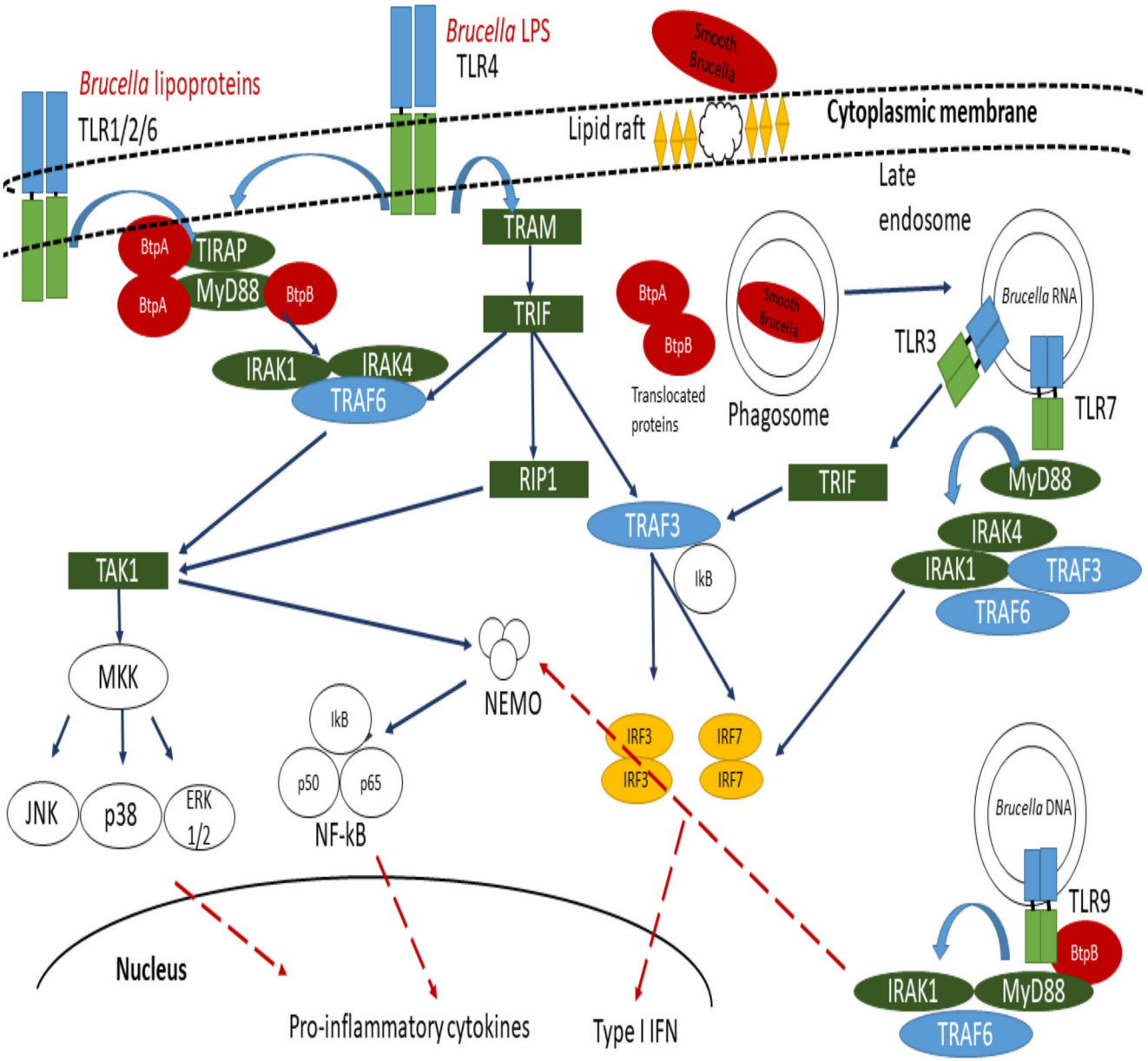

Fig. 1 Schematic representation of *Brucella*-DC interaction. Lipid rafts-mediated interaction between *Brucella* and DCs has been reported. TLR2, TLR4, TLR6, TLR3, TLR7, and TLR9 have been involved in *Brucella* recognition. However, *Brucella* phagocytosis involved recruitment of TLR2 but not TLR4 at the cytoplasmic membrane, and almost 90% of the ingested *Brucella* are eliminated by professional phagocytes; the fusion of late endosomes with intracellular receptors such as TLR9 allows type I IFN induction. Once inside, *Brucella* survive inside phagocytic vacuole and evade late endosomal traffic to reach intracellular niche. *Brucella* TIR proteins are translocated into cytoplasmic compartment. BtpA impairs DC activation/maturation through MyD88 and TIRAP interaction (Chaudhary et al. 2012; Radhakrishnan et al. 2009; Sengupta et al. 2010), and BtpB binds to various eukaryotic TIR-proteins (TLR2, TLR4, TLR9 MyD88, TIRAP, etc.) (Salcedo et al. 2013). Moreover, *Brucella* impairs type I Interferon family expression. Blue solid arrows indicate intracellular pathways activated via *Brucella* recognition by TLRs. Red dashed arrows indicate impaired cytokine pathways by *Brucella* TIR proteins and subsequent DCs maturation

performed with a purified protein, purified LPS, heat-killed bacteria or a live mutant strain is not the same. For example, in the case of subunit stimuli, although they are fundamentally important to determine the role of a specific macromolecule, they do not contain all elements encompassed by the whole bacterium and act together at the time of infection. However, some variables can be controlled, such as the dose and time of infection/stimulation with *Brucella* or *Brucella* antigens, the time of DC subset differentiation, the strain and the age of the animals used.

Thanks to studies of the interaction between *Brucella* and DCs, it has been possible to identify important virulence factors that may be key therapeutic targets for the control of brucellosis, for example, the proteins BtpA and BtpB, the core

region of LPS or the *wadC* gene. These elements fundamentally affect the immune response induced by dendritic cells.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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