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Review

Evasion of host defense by *Brucella*

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ABSTRACT

Brucella, an adept intracellular pathogen, causes brucellosis, a zoonotic disease leading to significant global impacts on animal welfare and the economy. Regrettably, there is currently no approved and effective vaccine for human use. The ability of *Brucella* to evade host defenses is essential for establishing chronic infection and ensuring stable intracellular growth. *Brucella* employs various mechanisms to evade and undermine the innate and adaptive immune responses of the host through modulating the activation of pattern recognition receptors (PRRs), inflammatory responses, or the activation of immune cells like dendritic cells (DCs) to inhibit antigen presentation. Moreover, it regulates multiple cellular processes such as apoptosis, pyroptosis, and autophagy to establish persistent infection within host cells. This review summarizes the recently discovered mechanisms employed by *Brucella* to subvert host immune responses and research progress on vaccines, with the aim of advancing our understanding of brucellosis and facilitating the development of more effective vaccines and therapeutic approaches against *Brucella*.

1. Introduction

Brucellosis is a significant zoonotic disease caused by *Brucella* species, widely distributed worldwide (Buttigieg et al., 2018; Godfroid et al., 2005). In human, *Brucella* infection leads to symptoms including undulant fever, arthritis, endocarditis, and meningitis, with a prolonged and recurring course that negatively impacts workforce productivity. In animals, brucellosis results in abortion and infertility, posing substantial threats and economic losses to both public health and the agricultural industry (Moreno, 2014). Notably, the disease is prevalent in pastoral regions and has gradually spread from occupational to non-occupational populations in recent years during the transition from pastoral to non-pastoral areas (Pappas, 2010; Pappas, Papadimitriou, Akritidis, Christou, & Tsianos, 2006). At present, there is no internationally recognized safe and effective human vaccine against *Brucella* (Heidary et al., 2022).

Brucella is an aerobic, gram-negative bacteria that exhibits a partially intracellular parasitic form, characterized by a short or coccobacillary morphology (Godfroid et al., 2005). Based on variances in pathogenicity and host specificity, the genus *Brucella* is classified into 6 species and 19 biotypes, with *B. melitensis*, *B. abortus*, and *B. suis* posing significant threats to human (Eisenberg et al., 2012; Eisenberg et al., 2017; Im, Jung, Shin, Kim, & Yoo, 2016). *Brucella*'s pathogenicity mainly stems from its ability to evade host defense system and survive long-term within specialized phagocytes such as macrophages and dendritic cells (DCs), as well as placental trophoblast cells (Copin et al., 2012; Martirosyan & Gorvel, 2013). The virulence factors of *Brucella* include lipopolysaccharide (LPS), flagellum, the type IV secretion system (T4SS) and outer membrane proteins (Omps) (Coloma-Rivero et al., 2021; Gorvel & Moreno, 2002; Roop, Barton, Hopersberger, & Martin, 2021). The T4SS secrets effector proteins into host cells to evade host immune responses and promote *Brucella* replication, leading to persistent chronic infections in host cells (Xiong et al., 2021). T4SS is conserved in all *Brucella* species and its expression is regulated by environmental signals, such as low pH, high temperature, and interaction with host cells (Ke, Wang, Li, & Chen, 2015; Sieira, Comerci, Sánchez, & Ugalde, 2000). The secretion apparatus of *Brucella*'s T4SS is comprised of three main components: the core components (VirB3, VirB4, VirB6-10), the external pilus (VirB2 and VirB5), and energy-providing ATPases (VirB4, VirB11), which form a translocation channel allowing effector proteins to be delivered into host cells. While a significant number of *Brucella* effector proteins have been identified and studied, the exact count of effector proteins remains to be fully determined. Currently, 15 effector proteins have been identified to manipulate host cellular processes and immune responses, including RicA, VceC, VecA, BtpA, BtpB, BspA, and BspB (Lacerda, Salcedo, & Gorvel, 2013; Lacerda et al., 2013; Xiong et al., 2021). Ongoing research

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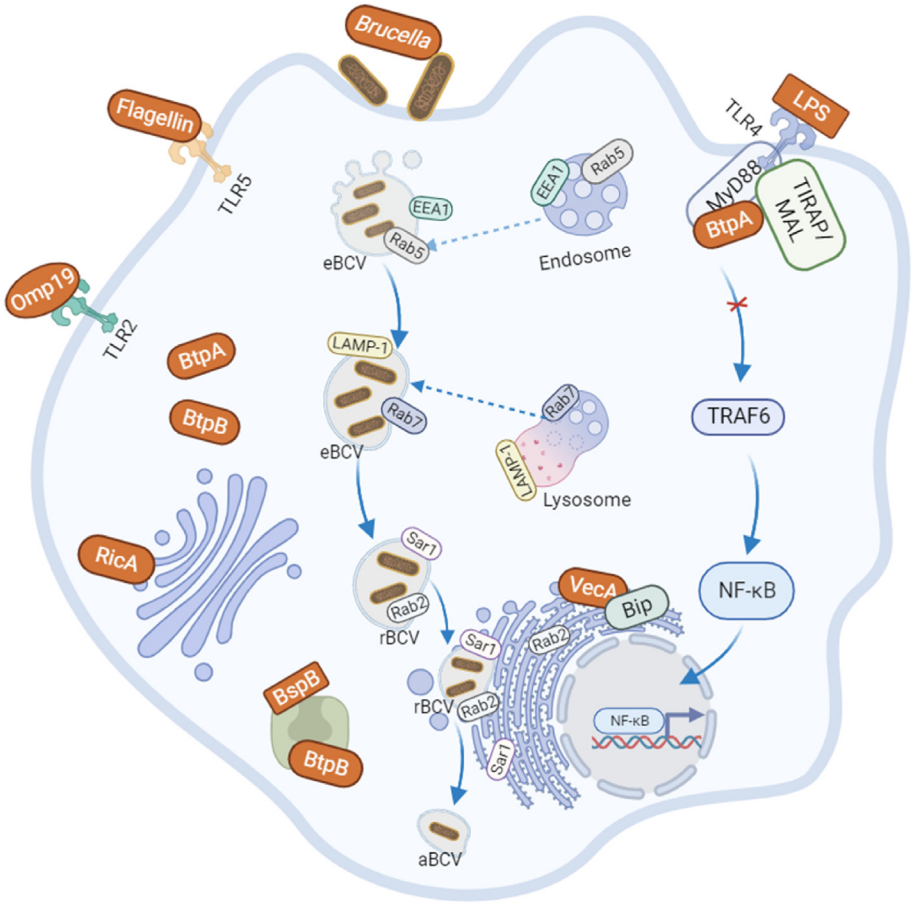

Fig. 1. The replication cycle of *Brucella* and potential mechanisms to evade innate immunity. *Brucella* binds to the host cell membrane using lipid rafts and utilizes intracellular vesicles for transportation. Once inside the host cell, it forms a vesicle called the *Brucella*-containing vacuole (BCV). The BCV then acquires certain host marker molecules (EEA1 and Rab5), which transforms it into an early BCV (eBCV). The eBCV interacts with lysosomes, acquiring additional host marker proteins (Rab7 and LAMP-1) to facilitate the fusion between the eBCV and lysosomes, allowing *Brucella* to evade the damaging effects of lysosomes. Through T4SS, an effector protein interacts with the exit site of the ER, enabling the eBCV transition to the ER, where it acquires marker molecules (Rab2 and Sar1), resulting in the formation of rBCV. The ER provides an optimal environment for *Brucella* replication. Ultimately, *Brucella* establishes colonization within the ER, undergoes proliferation, and is eventually released, initiating a new round of pathogenic infection. Several effector proteins secreted by *Brucella*'s T4SS are crucial for suppressing the host immune response. BtpA competitively binds to TIRAP/MAL, which prevents TIRAP/MAL from interacting with MyD88 and inhibits the NF-κB signaling pathway activated by TLR4, enabling *Brucella* to evade the innate immune response. *Brucella* VceC is involved in endoplasmic reticulum stress. The arrowhead represents the directional translocation of vesicles and signal transduction.

aiming to discover and characterize additional effector proteins to enhance our understanding of host-pathogen interactions is crucial for therapy and vaccines development targeting *Brucella*.

The immune system comprises innate immunity and adaptive immunity (Li, 2022; Zhong, Tien, & Shu, 2006). Innate immunity serves as the initial defense against pathogen intrusion and relies on the recognition of pathogen-associated molecular patterns (PAMPs) by host pattern recognition receptors (PRRs) to trigger the production of interferons (IFNs) and inflammatory cytokines that eliminate pathogens from the host (Hu & Shu, 2018; Xia, Yi, Wu, Shang, & Shu, 2019; Yi, Lian, & Li, 2022; Zhong et al., 2006). Adaptive immunity serves as a secondary defense mechanism to innate immunity by activating antigen-presenting cells such as dendritic cells, phagocytes, cytotoxic lymphocytes, and promoting the production of antibodies (An et al., 2022; Li, 2022; Ruf, Greten, & Korangy, 2023; Shao, Liu, & Qi, 2023). During prolonged interactions with the host, *Brucella* undergoes evolutionary adaptations and primarily employs "stealth" strategies to evade, interfere with, or suppress immune responses, leading to the establishment of chronic and persistent infection (Cardoso, Macedo, Azevedo, & Oliveira, 2006; Lacerda et al., 2013; Lapaque, Muller, Alexopoulou, Howard, & Gorvel, 2009). This review summarizes the recently discovered mechanisms employed by *Brucella* to subvert host immune responses and cellular homeostasis, as well as reveals research progress on vaccines with the aim of advancing our understanding of brucellosis and facilitating the development of more effective vaccines and therapeutic approaches against *Brucella*.

1.1. The cellular niche of Brucella

Brucella has a strong ability to persist and reproduce within macrophages, dendritic cells, and placental trophoblast cells (Copin et al., 2012). *Brucella* infection can be divided into three stages: invasion, acute infection, and chronic infection. At the beginning of the infection, host cells activate innate immunity to suppress *Brucella* replication, while adaptive immunity takes over during the later stages (Baldwin & Goenka, 2006). Nevertheless, *Brucella* employs various strategies to evade the host's immune system, allowing for colonization, growth, and prolonged multiplication. The chronic infection occurs due to the balanced interaction between *Brucella* and the host to maintain over a long period of time (Grilló, Blasco, Gorvel, Moriyón, & Moreno, 2012).

To establish infection, *Brucella* initially interacts with host cell membranes through lipid rafts, facilitating its intracellular vesicular transport by forming a specialized compartment called the *Brucella*-containing vacuole (BCV). The BCV temporarily associates with endosomes forming an early endosomal *Brucella*-containing vacuole (eBCV). During transportation, eBCVs lose early endosomal markers, and most of them are cleared by lysosomes but a small number of eBCVs evade lysosomal clearance. Studies suggest that the fusion of eBCV with lysosomes can promote its maturation and connection with the endoplasmic reticulum (ER) (Starr, Ng, Wehrly, Knodler, & Celli, 2008). At this stage, the eBCV transforms into a mature replicating *Brucella*-containing vacuole (rBCV). The ER provides an optimal environment for *Brucella* to replicate and enhance its pathogenicity (Sedzicki et al., 2018). Current studies suggest that eBCV can merge with the ER via COP II vesicles and activate Sar1 protein during vesicle transport (Celli, 2019; Jiao et al., 2021). Additionally, the fusion of eBCV with the ER is dependent on the small GTPase Rab2 (Fugier et al., 2009). Yip1A phosphorylates IRE1α at the ERES, leading to the activation of IRE1α. This activation stimulates the production of ER-derived vesicles that merge with eBCV, resulting in the formation of rBCV, and this continuous fusion process with secreted ER vesicles facilitates *Brucella* proliferation (Taguchi et al., 2015). Whereas the precise molecular mechanisms mediating the fusion of eBCV

Table 1
Brucella encoded proteins involved in evading host innate immunity.

Name	Gene	Type	Function	Seleted references
Omp19	BS1330_I1924	Omps	Inhibit MHC-II expression and antigen processing by interacting with TLR2.	Delpino et al., 2012; Ferrero et al., 2014
			Evade the proteolytic defense system.	Pasquevich et al., 2019
Omp22	Bsuis_A1680	Omps	Inhibit the expression of inflammatory cytokines.	Xu et al., 2005
Omp25	BS1330_I0697	Omps	Inhibit the NF-κB signaling pathway and production of inflammatory cytokines.	Degos et al., 2020
			Degrade cGAS by ubiquitin proteasome pathway and inhibit IFN-β production.	Li et al., 2021
			Regulate miRNA to inhibit TNF-α production.	Cui et al., 2017
			Inhibit the maturation of DCs and reduce the activity of antigen presentation.	Zhang et al., 2017b
Omp31	BRA0423	Omps	Inhibit NF-κB p65 signal pathway and TNF-α expression.	Wang et al., 2021; Zhang et al., 2016
TcpB/ BtpA	BAB1_0279	Secretory	Inhibit the NF-κB signaling pathway mediated by TLR4 and TLR2 and the maturation of DCs.	Salcedo et al., 2008; Saqib & Baig, 2019
			Combine with MyD88 and degrade MAL.	Sengupta et al., 2010
			Inhibit $CD8^+$T cells from killing infected bacterial cells.	Durward et al., 2012
			Inhibit the activation of inflammasome.	Jakka et al., 2017
BtpB	BAB1_0756	Secretory	Inhibit of signal transduction of TLR2 and TLR4 and inflammatory. Interaction with MyD88 and regulation of NF-κB translocation to the nucleus.	Salcedo et al., 2013
BspB	BAB1_0712	Secretory	Competitive binding of IRF3, inhibition of phosphorylation and nucleation of IRF3, and inhibition of IFN-β production.	Li, 2023
RicA	BMEI_0736	Secretory	Degrade STING by the autophagosome pathway and inhibit IFN-β production.	Li, 2023
VceC	BAB1_1058	Secretory	Activate NF-κB signaling pathway and participate in inflammatory reaction.	De Jong et al., 2013
			Induce unfolded protein reaction to recombine the structure of ER.	De Jong et al., 2013; Zhi et al., 2019
c-di-GMP		Second messenger	Interact with STING, and initiate IFN-I immune response via TBK1-IRF3 signaling pathway.	Costa Franco et al., 2018 Roop et al., 2021
LPS		Lipopolysaccharide	Evade TLR4 identification.	Lapaque et al., 2006
			Inhibit the activation of the complement system.	Barquero-Calvo et al., 2007
Flagellin			Evade TLR5 identification.	Andersen-Nissen et al., 2005

with the ER have yet to be fully elucidated and require further investigation. In later stages of infection, rBCV transforms into autophagic *Brucella*-containing vacuoles (aBCV), which differ from traditional autophagosomes. Intriguingly, the formation of aBCV requires autophagy initiation factors like ULK1, Beclin1, ATG14L, and PI3K kinase, but it does not involve autophagy extension factors such as ATG5, ATG16L1, ATG4B, ATG7, and LC3 (Jiao et al., 2021; Starr et al., 2012). Eventually, *Brucella* completes its intracellular cycle and is released through lytic and non-lytic mechanisms, allowing for a new round of infection (Fig. 1).

Under acidic pH conditions, the *Brucella* type IV secretion system can be triggered to release various effector proteins, which aid *Brucella* in evading host defense responses and suppressing the host immune responses (Boschiroli et al., 2002). In recent years, various studies have revealed that *Brucella*, as well as other intracellular parasitic pathogens like Chlamydia, employ different strategies to exploit the vesicular transport pathway of host cells (Bestebroer, V'kovski, Mauthe, & Reggiori, 2013; Chen, Zhao, & Zhang, 2022). These findings suggest the potential for developing vaccines that can stimulate immune responses to interfere with the initial stages of infection, including neutralizing the virulence factors released by *Brucella* to inhibit its replication within host cells.

1.2. *Brucella undermines innate immunity*

The innate immune response serves as the initial defense against pathogens infection, playing a crucial role in inhibiting and eliminating invading pathogens (Wu et al., 2019). This defense involves the coordinated actions of immune cells like macrophages, immune molecules like PRRs and PAMPs, and complement systems (Martirosyan, Moreno, & Gorvel, 2011). PRRs comprise multiple components, including Toll-like receptors (TLRs) located on the cell membrane, intracellular Nod-like receptors (NLRs), RNA or DNA sensors and the complement system (Zhang & Zhong, 2022). These receptors recognize PAMPs and initiate appropriate immune responses to restrain pathogens infection to enhance host defense responses (Martirosyan et al., 2011; Shu, Takeuchi, & Goeddel, 1996; Wu et al., 2022; Xu et al., 2005; Zheng et al., 2022; Zhong et al., 2008). On the other hand, *Brucella* employs "stealth" strategies to evade host immune responses, specifically by evading continuous recognition by PRRs and suppressing immune responses. These strategies allow *Brucella* to effectively evade and subvert host immune defenses (Martirosyan et al., 2011).

1.3. *Brucella evades PRRs recognition*

During *Brucella* infection, innate immune responses are not effectively triggered due to unique structural characteristics of *Brucella* which can prevent the activation of PRRs and the complement system. TLRs are crucial regulators of innate immune signaling and facilitate communications between adaptive and innate immunity, allowing the immune system to mount strong responses against various pathogens (Yang et al., 2017; Zhong et al., 2020). The molecular components on the surface of *Brucella*, such as lipopolysaccharides (LPS), lipoproteins, and flagella, do not effectively stimulate the innate immune system by evading PRRs recognition (Gorvel, 2008). The lipid A of *Brucella* LPS has longer fatty acid chains (C28), which reduces its recognition by TLR4 and suppresses the activation of inflammatory response (Lapaque et al., 2006). However, the flagellin produced by *Brucella* lacks the specific structural domain that activates TLR5, further contributing to its ability to evade immune detection (Andersen-Nissen et al., 2005; Hoebe, Janssen, & Beutler, 2004) (Fig. 1). To successfully replicate, *Brucella* has developed the T4SS which secretes effector proteins to manipulate immune signaling, facilitating adhesion, internalization, intracellular transport, and replication within host cells (Ke et al., 2015; Xiong et al., 2021). Notably, BtpA and BtpB, important virulence factors, possess conserved Toll/Interleukin 1 receptor (TIR) structural domains. These domains can regulate innate immune responses by inhibiting the activation of the TLR signaling pathway (Lacerda et al., 2013). The TIR domain containing adapter protein (TIRAP/MAL) mediates TLR signaling pathway through interaction with MyD88 (Li et al., 2016; O'Neill et al., 2003). BtpA competitively binds to MyD88 and promotes the degradation of phosphorylated TIRAP/MAL by enhancing its polyubiquitination, effectively blocking TIRAP/MAL-induced NF-κB activation (Sengupta et al., 2010; Snyder et al., 2014). On the other hand, BtpB exhibits an even stronger inhibition of TLR2, TLR4, and TLR9 signaling compared to BtpA. BtpB also interacts

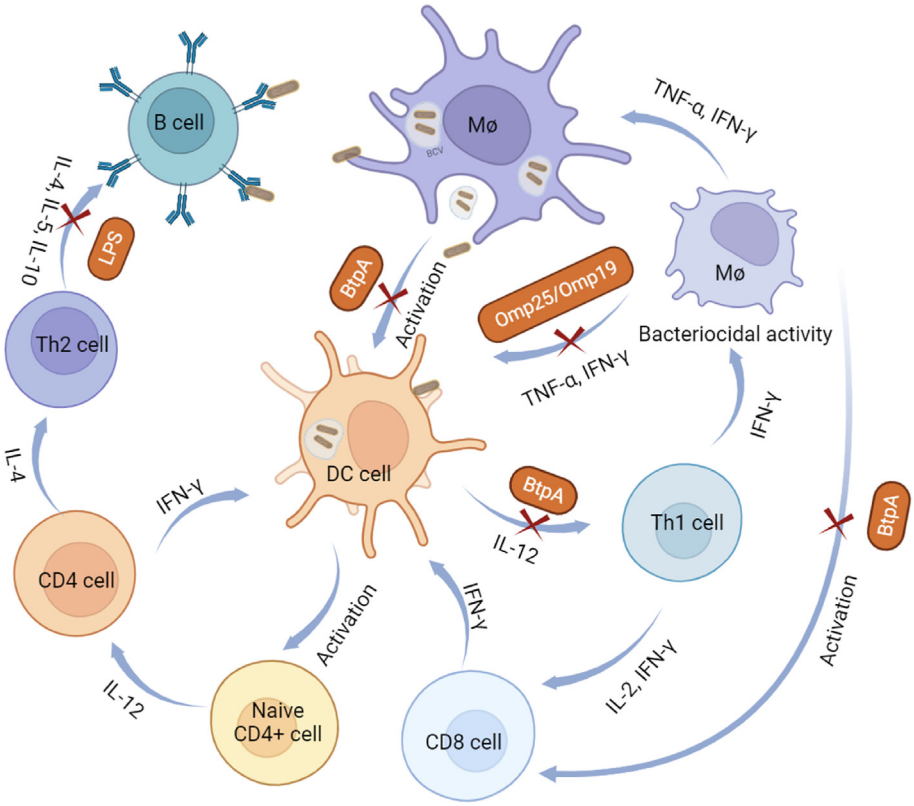

Fig. 2. *Brucella* modulates adaptive immune responses. Recognition of *Brucella* antigens by antigen-presenting cells (APCs) leads to the production of IL-12 cytokines. These cytokines activate $CD4^+$ T cells and Th2 helper T cells, which secrete cytokines like IL-4 and IL-10. These cytokines further stimulate B lymphocytes, enhancing their involvement in humoral immunity and facilitating the rapid elimination of pathogens from the host. Additionally, APCs can activate Th1 helper T cells, promote the maturation of $CD8^+$ T cells, and participate in cytotoxic T cell (CTL) activity. This CTL activity specifically targets and destroys *Brucella*-infected cells, while also releasing various cytokines such as TNF-α, IL-2, and IFN-γ. These cytokines play a role in promoting the immune clearance of *Brucella* by macrophages.

with MyD88 and prevents NF-κB translocating to the nucleus (radhakrishnan & Splitter, 2010; Salcedo et al., 2013; Saqib & Baig, 2019). Recently, studies have shown that NLRs, as intracellular PRRs, can also recognize intracellular *Brucella* (Taguchi et al., 2015).

1.4. *Brucella evades the inflammatory signaling*

The inflammatory responses serve as crucial defense mechanisms for eliminating pathogens infection and initiating the healing process (Hsu, Shu, Pan, & Goeddel, 1996; Lei et al., 2019). Multiple outer membrane proteins (Omps) regulate inflammatory responses and promote self-replication. Notably, Omp25 and Omp31 are significant Omps associated with *Brucella*'s virulence, with the deletion of Omp25 shown to decrease virulence and promote the production of inflammatory factors in murine models (Yang et al., 2020). Omp25 has been found to upregulate microRNAs associated with TNF-α, inhibiting TNF-α production and suppressing the release of inflammatory factors mediated by the NF-κB pathway (Luo et al., 2017). Additionally, Omp25 can modulate the *Brucella*-activated MAPK pathway, further suppressing TNF-α expression (ZHANG, ZHANG, et al., 2017). Omp31 plays a critical role in maintaining the integrity of *Brucella*'s outer membrane, which is essential for bacterial invasion, establishment, and the formation of replicative ecological niches (BAI et al., 2021). Omp31-induced autophagy negatively regulates the NF-κB p65 signaling pathway, leading to the inhibition of TNF-α expression (Wang et al., 2021).

1.5. *Brucella evades the complement system*

The complement system is a diverse group of proteins that can effectively kill most Gram-negative bacteria by binding to C3 and initiating complement system-mediated clearance (Hoffmann & Houle, 1995). The O-antigen of *Brucella* is distinct from that of *Salmonella typhimurium*. Specifically, the O-antigen of *Brucella* lacks free OH-groups and is linked to 4,6-dideoxy-4-formamido-alpha-d mannopyranosyl residues, enabling *Brucella* to hinder the production of C3a and C5a upon interaction with C3, thereby modulating the host immune response. Furthermore, *Brucella*'s surface lipopolysaccharide (LPS) has long polysaccharide side chains that make it difficult for the complement factors to bind to the bacteria's cell membrane. As a result, *Brucella* can escape recognition and destruction by the complement system (Barquero-Calvo et al., 2007).

These studies suggest that *Brucella* employs specific strategies including modulating phagocytic activity, inhibiting PRRs like TLRs and NLRs, suppressing inflammatory responses, and interfering with the complement system to regulate and evade the innate immune responses (Table 1). Furthermore, the Table 1 also provides a summary of *Brucella* virulence factors involved in the interaction between *Brucella* and the host's innate immunity.

1.6. *Brucella undermines adaptive immunity*

Adaptive immunity is a defense mechanism in the immune system that develops long-term immunity against infections (Cooper & Alder, 2006; Netea, Schlitzer, Placek, Joosten, & Schultze, 2019). When *Brucella* infection occurs, three primary mechanisms activate the adaptive immune response (Martirosyan & Gorvel, 2013). Firstly, $CD4^+$/$CD8^+$ T cells produce IFN-γ, activating macrophages to prevent *Brucella* from replicating inside host cells. A second mechanism involves $CD8^+$ T cells, which have a cytotoxic effect that eliminates infected macrophages. Additionally, the third mechanism involves Th1 antibody subtypes like IgG2a/IgG3, which enhance the phagocytosis of degraded BCVs (Grilló et al., 2012; Martirosyan & Gorvel, 2013). Antigen-presenting cells (APCs) recognize *Brucella* antigens and secrete IL-12 cytokines. These cytokines activate $CD4^+$ and $CD8^+$ T cells, as well as Th1 and Th2 helper T cells. In turn, these cells secrete cytokines like TNFα, IL-2, IL-4, IL-10, and IFN-γ, which help regulate the adaptive immune response and aid in the clearance of *Brucella* by macrophages (Fig. 2) (Elrashedy et al.; Ali et al., 2023; Martirosyan et al., 2011). To establish a persistent infection

in the host, *Brucella* steadily exerts immunosuppressive effects on the adaptive immune response. In mice infected with *Brucella* abortus, there is an increased population of $CD4^+$ and $CD25^+$ T cells in the spleen which play a regulatory role in the persistence of the infection (Pasquali et al., 2010). *Brucella* has developed mechanisms to hinder the transition from innate to adaptive immune signaling by manipulating DCs, thereby evading host immunity (Dudek, Martin, Garg, & Agostinis, 2013). *Brucella* Omp19 and Omp25 have been shown to reduce the secretion of cytokines TNF-α and IL-12, which subsequently impairs the activation of T-cells by DCs. This hindrance in initiating the host's protective Th1 immune responses gives *Brucella* an advantage in effectively concealing their presence (Avila-Calderón et al., 2020; Billard, Dornand, & Gross, 2007; Martirosyan et al., 2011; Salcedo et al., 2008). *Brucella* LPS can evade host adaptive immune clearance by impairing the recognition and presentation of antigens by MHC II molecules in APCs and inhibiting antibody production by B cells (Lapaque et al., 2006). Recent studies have also found that BtpA, a novel effector involved in evading the adaptive immune response, interacts with phosphatidylinositol-4, 5-bisphosphate (PI (4,5) P2) or PI (3,4,5) P3. This interaction inhibits the activation of APCs at the immune synapse, impairing the cytotoxicity of $CD8^+$ T cells (Durward et al., 2012).

In summary, the inadequate immune responses seen in *Brucella* infection can be attributed to both the inhibition of innate immune responses and adaptive immune responses, which struggle to fully eradicate the infection. Improving our understanding of the interaction between *Brucella* and the host's immune system could provide valuable insights into *Brucella*'s pathogenesis and aid in the development of effective vaccines and therapies.

1.7. *Brucella* regulates cellular homeostasis

Cellular homeostasis is vital for maintaining cell functions, including stability, metabolism, and response to environmental stresses. Loss of control over cellular homeostasis can lead to diseases like cancer, inflammation, and immune system disorders (Meizlish, Franklin, Zhou, & Medzhitov, 2021; Wei, Zheng, Chapman, Geiger, & Chi, 2021; Wong et al., 2021; Yin, Chen, & Eisenbarth, 2021; Zhang et al., 2021). The endoplasmic reticulum (ER) serves as the calcium reservoir and is responsible for processing, folding, and transporting proteins, crucial for maintaining cellular homeostasis (Marchi et al., 2018). *Brucella* invades the cell and proliferates within the ER, disrupting its homeostasis and inducing prolonged and excessive endoplasmic reticulum stress (ER Stress). This disruption fails to restore cellular homeostasis, ultimately causing cell death (Byndloss et al., 2019; Wang et al., 2015, 2016). Furthermore, *Brucella* can evade the host elimination by inhibiting cell death and utilize the host autophagy process.

1.8. *Brucella* regulates apoptosis

Apoptosis is a regulatory mechanism responsible for programmed cell death and defending against intracellular bacteria, mainly triggered by caspases (Bronner, O'Riordan, & He, 2013). *Brucella* evades the immune response by inhibiting apoptosis, enabling the bacteria to replicate within infected cells. This is partially achieved by increasing the expression of zinc finger protein A20, which inhibits the NF-κB pathway. As a result, Caspase-8 activation caused macrophage apoptosis is restricted to create a favorable environment for bacterial proliferation (Wei et al., 2015). Additionally, *B. melitensis* infection has been found to reduce the expression of genes involved in the mitochondrial apoptotic pathway. *Brucella* VceC regulates IRE1 pathway and inhibits CHOP-induced apoptosis to support replication in goat trophoblast cells (Zhi et al., 2019). These findings collectively indicate that *Brucella* employs apoptosis inhibition as a strategy to evade the host immune response and promote intracellular replication.

1.9. *Brucella* regulates pyroptosis

Pyroptosis, also known as cellular inflammatory necrosis, is an important immune response against pathogens. It is triggered by the activation of caspase-1 and leads to the release of pro-inflammatory cytokines like IL-1β and IL-18 to help recruit and activate immune cells to control infections (Hsu et al., 2021). Key mediators such as Caspase-1, Gasdermin D (GSDMD), IL-1β, IL-18, NLRP3, AIM2, and others play a crucial role in protecting against bacterial infections (Karmakar et al., 2020; Marim et al., 2017; Shi et al., 2021). Extensive research has shown that pyroptosis acts as a defense mechanism against *Brucella* infections (Costa Franco et al., 2019; Lacey, Mitchell, Dadelahi, & Skyberg, 2018; Tupik et al., 2020). To persist within host cells, *Brucella* employs various strategies to inhibit pyroptosis. Recent studies have revealed that the T4SS plays a critical role in triggering the formation of inflammatory vesicles in infected macrophages (Marim et al., 2017). The secreted protein TcpB, derived from *Brucella*-infected macrophages, inhibits the activation of nonclassical inflammatory vesicles induced by LPS. Moreover, TcpB induces ubiquitination and subsequent degradation of caspase-1, caspase-4, and caspase-11, effectively blocking pyroptosis (Jakka, Namani, Murugan, Rai, & Radhakrishnan, 2017). These findings indicate that *Brucella* actively interferes with cell death. Importantly, further investigation is needed to fully understand the underlying mechanism.

1.10. *Brucella* regulates autophagy

Autophagy is a significant defense process employed by the host to remove intracellular pathogens. Some bacteria have the ability to manipulate autophagy, which helps them survive and evade host clearance (Liu & Levine, 2015). Studies have demonstrated that autophagy-associated proteins play a vital role in the formation of rBCV, aiding *Brucella* in completing its intracellular life cycle (Starr et al., 2012). By activating IRE1α through Yip1A, *Brucella* upregulates the expression of Sar1 and COPII, leading to vacuole formation in the endoplasmic reticulum. This conversion of BCV to rBCV promotes *Brucella*'s survival (Taguchi et al., 2015; Wang et al., 2014). These findings highlight how *Brucella* manipulates autophagy as a strategy to counteract host immune responses. Identifying and targeting *Brucella* effectors or host molecular switches involved in regulating defense responses could provide a novel approach to controlling *Brucella* infections.

1.11. *Brucella* vaccines

Vaccination is one of the most effective strategies in controlling *Brucella* infection. However, there is currently no globally recognized vaccine against *Brucella* for human use (Jiang, O'callaghan, & Ding, 2020). Moreover, there is few information and relevant data regarding clinical trials of *Brucella* vaccines in human subjects. The key challenge in developing *Brucella* vaccine for human use are safety issues, which must strike a delicate balance between safety and efficiency (Vershilova, 1961). In addition, the pathogenesis of *Brucella* and the mechanisms by which it escapes host immune responses and perpetuates infection are not fully understood. Moreover, the uniqueness and complexity of *Brucella* itself caused by multiple strains and genetic variants that allow it to evade and manipulate the host immune system, posing a significant obstacle to vaccine development (Masjedian Jezi, Razavi, Mirnejad, & Zamani, 2019). Given the consistent incidence of human brucellosis, it is imperative to sustain research efforts focused on the development of human vaccines that are both safe and effective, while also providing cross-protection against various strains of *Brucella*.

The management of human brucellosis has greatly depended on controlling animal brucellosis through effective vaccination strategies. Live attenuated vaccines, such as *B. abortus* S19/A19, *B. melitensis* Rev1, and *B. suis* S2, are widely used to control brucellosis in animals. While these vaccines have been successful in producing long-lasting antibodies,

Table 2
Brucella vaccines.

Type	Name of vaccine/Antigens	Vector	Advantage	Disadvantage	References
Live-attenuated vaccines	*B. abortus* strains S19/*A19* *B. melitensis strain Rev1* *B. suis* strain S2	No	High and wide immunoprotective capacity	Interference with serological diagnosis; Limited protective host range; Antibiotic resistance.	Truong et al. 2015 Verger et al., 1995; Zhu et al., 2016
	B. abortus (△cydC, cydD)/(△cydC1, purD)/(△norD, znuA)	No	Induction of significant levels of IgG antibodies; Induction of higher levels of IFN-γ, IL-10, IL-4 and TNF-α; Provides security and stability.	Risk of reintensification of vaccine strains	Li et al., 2017b, 2017c; Truong et al., 2016
	B. melitensis 16M △*hfq*/△ TcfSR/△ mucR	No	Induction of significant levels of IgG antibodies; Induction of higher levels of IFN-and IL-4; Reduced pathologic damage.		Arenas-Gamboa, Rice-Ficht, Kahl-Mcdonagh, & Ficht, 2011; Lei et al., 2015; Li, Zhang, et al., 2015; Zhang et al., 2013
	B. melitensis M5-90 △*bp26*/△*vjbR*/△ *manB*/△wboA	No	Reduced virulence and higher level of immune protection; Induction of significant levels of IgG antibodies; Induction of higher levels of IFNs and IL-4; Ability to differentiate between serotypes of infected and vaccinated animals.		Li, Shi, et al., 2015; Li, Tong, et al., 2017; Li et al., 2018; Zhang, Yin, et al., 2017
	B. suis △*pgm*	No	Induces strong cellular immune responses and pro-inflammatory factors production; virulence reduction.		Czibener, Del Giudice, Spera, Fulgenzi, and Ugalde, 2016
Subunit vaccines	Omp25-Omp31	No	Induction of higher levels of IFN-γ and TNF-α; Activation of Th1 type immune response.	Low levels of protective antibodies	Mohammadi, 2021
	Omp25-L7/12	No	Induction of significant levels of IgG antibodies; Activation of Th2 type immune response.		Gupta, Mohan, Somani, Aggarwal, and Bhatnagar, 2020
	Omp16/19/28-L7/12	No	Induction of higher levels of TNF-α, IL-6 and MCP-1; Activation of Th1 type immune response.		Huy et al., 2020
	Omp31、BP26、BLS、DnaK and L7/12	No	dominant antigen epitope vaccine (DAEV); Induction of significant levels of IgG antibodies; Induction of higher levels of TNF-α , IFN-γ and IL-10; Induction of mixed Th1 and Th2 immune responses.		Gupta, Singh, Gupta, and Bhatnagar, 2019
Vector vaccines	Omp19, Cu-Zn superoxide dismutase (SOD)	*S. typhimurium*	SOD induces high level of IFN-γ expression; Omp19 induces the production of higher titers of IgG antibodies; Induction of mixed Th1 and Th2 immune responses.	Presence of potentially pathogenic antigens	Hewawaduge et al., 2020
	Omp 16/19, L7/12, and Cu-Zn SOD	Influenza viral vector (rIVV) subtype H5N1	Immunization effects similar to live attenuated vaccines	Presence of potentially pathogenic antigens;	Bugybayeva et al., 2020
	L7/12 and BCSP31	Adenovirus	Induction of significant levels of IgG antibodies; Induction of higher levels of IL-12 and IL-10.	Weaker than live attenuated A19 vaccine; Presence of potentially pathogenic antigens	Vellinga et al., 2014
	OMP19	*L. casei*	Induction of higher levels of IFN-γ、IL-2 and IL-4; Activation of Th1 and Th2 type immune response.	Presence of potentially pathogenic antigens	Mohammadi and Golchin, 2020
	Cu-Zn SOD	*L lactis*	Induces mucosal type 2 immune responses; SOD induces systemic and mucosal specific immune responses.		Sáez, Fernández, Rivera, Andrews, and Oñate, 2012
DNA vaccines	Omp25-Omp31	pcDNA3.1	Flexible design and high security; Induces the production of high levels of IgG antibodies and IFN-γ;	Lower levels of antibody protection	Shojaei, Tahmoorespur, Soltani, and Sekhavati, 2018

(*continued on next page*)

Table 2 (*continued*)

Type	Name of vaccine/ Antigens	Vector	Advantage	Disadvantage	References
			Activates strong humoral and cellular immunity.		
	Cu-Zn SOD	pcDNA	DNA epitope vaccines; Flexible design and high security; Induction of high levels of IgG antibodies, IFN-γ and Th1 immunoreactivity.	No induction of IL-4 production and Th2 immune response; Lower levels of antibody protection.	Escalona, Sáez, and Oñate, 2017
	BvrR	No	Flexible design and high security; Activation of Th1 type immune response. Induction of IFN-γ expression and T cell proliferation.	Low antibody titer	Chen, Liu, Zhao, and Wang, 2019
Nanoparticle based vaccines	Chimeric antigen TF/Bp26/Omp31 (TBO)	Glycine nanoparticles	Induce high levels of IgG and IgA antibodies; Induction of cellular and humoral immune responses.	Low levels of antibody protection	Karevan, Ahmadi, Taheri, and Fasihi-Ramandi, 2021
	Malate dehydrogenase (rMdh), Omp 10/19	Carbon nanosheet (CNs)	Induce increased expression of IgG and IgA antibodies, IFN-γ and IL-4; Activation of Th1 and Th2 type immune response.		Shim et al., 2020
	L7/L12	PLGA nanoparticles	Induction of high levels of IgG antibody titers and IFN-γ production; Activation of Th1 type immune response. Reduction of pathological damage		Singh, Somani, Aggarwal, and Bhatnagar, 2015
Other vaccines	bacterial-ghost (BG) 2308 GntR	No	High immunogenicity and safety; Induces high levels of IgG antibodies; Induction of increased expression of IFN-γ and IL-4; Promotes immune response of $CD4^+$ and $CD8^+$ T cells; Activation of Th1 type immune response	Lower lysis efficiency using Gram-negative bacteria as carriers	Liu et al., 2015; Wang et al., 2020
	L7/L12	*S. typhimurium*	Induction of significant levels of IgG antibodies; Induction of higher levels of TFN-γ and IL-4; Significant increase in both $CD4^+$ and $CD8^+$ cells; Lipopolysaccharide (LPS) present in the host produces specific immunity against Salmonella spp.		Senevirathne, Hewawaduge, and Lee, 2020

they have drawbacks such as interfering with serologic diagnosis, antibiotic resistance, and residual toxicity (Horwell & Van Drimmelen, 1971; Li, Shi, et al., 2015; Truong, Cho, Kim, Park, & Hahn, 2015; Verger, Grayon, Zundel, Lechopier, & Olivier-Bernardin, 1995; Zhu et al., 2016). In recent years, advances in molecular microbiology and genetic engineering have led to the development of new *Brucella* vaccines with equal or higher protective efficacy, including vaccines with live attenuated vaccines with gene deletion, subunit vaccines, DNA vaccines, and vector vaccines (Heidary et al., 2022). These vaccines aim to induce a strong immune response and have shown promising immunogenicity (Table 2). The proteins Omp31/28/25/19/16, ribosomal proteins L7/12, type IV secretion system-associated protein VirB12, and cytoplasmic binding protein P39 are strongly linked to the virulence and immunogenicity of *Brucella* (Heidary et al., 2022). Subunit vaccines offer non-infectious and cross-protective benefits but suffer from weak antigenicity, instability, and short half-life (Moyle & Toth, 2013). DNA vaccines are used to develop safe and effective *Brucella* vaccines, stimulating immune responses but not consistently providing protection (Velikovsky et al., 2000). Vector vaccines introduce antigen genes into attenuated bacteria or viruses to induce immunoprotective responses, such as with *S. typhimurium* and *Adenovirus* vectors (Hewawaduge, Senevirathne, & Lee, 2020; Vellinga et al., 2014). Although these vaccines enhance immunogenicity, they may carry pathogenic risks different from natural proteins. The live attenuated vaccines with virulence gene deletions show higher safety profiles compared to traditional vaccines, promoting T cell proliferation, pro-inflammatory cytokine expression, and antibody production, The live attenuated vaccines with virulence gene deletions hold promise as vaccine candidates for humans but need consideration regarding risks of reversion to virulence and antibiotic resistance (Perkins, Smither, & Atkins, 2010; Truong, Cho, Park, Kim, & Hahn, 2016). Additional factors to consider include the use of adjuvants, immunomodulators, and antigen-presenting systems to enhance immune response levels. Overall, the research and development of improved *Brucella* vaccines have garnered significant attention, offering potential alternatives to live attenuated vaccines with enhanced safety and effectiveness.

2. Conclusion

Brucella has developed potent mechanisms to avoid or undermine host defense mechanisms. *Brucella* displays stealthy methods of invading host cells, cleverly evading detection by PRRs and thereby bypassing immune surveillance. This interplay leads to a prolonged balance between *Brucella* and the host's immune defense, promoting its ability to survive within host cells for an extended period of time.

Understanding the complicated mechanism of *Brucella*-induced host immunosuppression remains a challenging task. *Brucella* not only evades detection to suppress the host's innate and adaptive immune responses but also impacts important cellular processes like apoptosis, pyroptosis, autophagy, and endoplasmic reticulum stress. These mechanisms allow *Brucella* to manipulate cellular functions, promoting its survival within the host. The virulence factors and effector proteins of *Brucella*'s T4SS play a crucial role in stabilizing *Brucella*-containing vacuoles within the endoplasmic reticulum, facilitating replication by manipulating or exploiting host proteins. Further research is needed to fully understand how *Brucella* utilizes these cellular processes for self-replication.

No vaccine against human brucellosis exists to date. Therefore, control of human brucellosis relies heavily on the control of animal brucellosis through vaccination. The most effective protection against *Brucella* invasion is provided by live attenuated vaccines (Sancho, Tejedor, Sidhu-Muñoz, Fernández-Lago, & Vizcaíno, 2014). While these vaccines produce long-lasting antibodies, they have drawbacks such as interfering

with standard serologic diagnostics, antibiotic resistance, and residual toxicity. The key to the development of more effective brucellosis vaccines is to induce a significant Th1 immune response and to provide a higher level of protection (de Figueiredo, Ficht, Rice-Ficht, Rossetti, & Adams, 2015; Pascual et al., 2018). In order to design and develop new strategies to enhance the immunogenicity of new vaccines, we still need to further elucidate the pathogenic mechanism of *Brucella*, and also need to conduct in-depth explorations on the selection of antigens, animal models, effective adjuvants and delivery vectors.

In summary, this manuscript provides a comprehensive review of *Brucella*'s pathogenic mechanism and its strategies to evade host defense responses. However, there is still much to explore regarding the specific factors responsible for host resistance to *Brucella* infection. Further exploration is needed to understand the influence of host genetic background, immune status, and physiological conditions on *Brucella* infection to help reveal individual differences in susceptibility and severity of infection, providing evidence for developing more personalized and precise therapies. Moreover, the differences in immune responses triggered by acute and chronic infections of *Brucella* are worth investigating, as these differences can have implications for interactions between *Brucella* and the host immune system, including the dynamics of host immune responses and differences in *Brucella* evasion strategies. This understanding can contribute to the development of more effective prevention and provide more precise treatment strategies for acute and chronic infections. Most importantly, *Brucella* has the ability to invade host cells and survive within them, yet the mechanisms underlying its intracellular survival remain incompletely understood. Therefore, further research is needed to elucidate how *Brucella* evades immune surveillance and clearance within host cells. Our understanding of the direct interactions between *Brucella*-secreted proteins and host proteins, which are crucial for *Brucella*'s ability to cause disease, is also limited. To further fulfill our knowledge, it is necessary to conduct additional studies using reliable screening systems such as affinity tag purification mass spectrometry, the transposon system, and the CRISPR-Cas9 screening system. These investigations will help us better understand the intricate molecular interactions between *Brucella* and the host, leading to the development of therapeutic agents and diagnostic tools for brucellosis.

Conflict of interest

The authors state that the manuscript was conducted without any commercial or financial relationships that could be considered as a potential conflict of interest.

Declaration of competing interest

The authors state that the manuscript was conducted without any commercial or financial relationships that could be considered as a potential conflict of interest.

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