Omp25-dependent engagement of SLAMF1 by *Brucella abortus* in dendritic cells limits acute inflammation and favours bacterial persistence *in vivo*

Clara Degos[1]*, Lisiena Hysenaj[1]*, Gabriela Gonzalez-Espinoza[1], Vilma Arce-Gorvel[1], Aurélie Gagnaire[1], Alexia Papadopoulos[1], Karina Alejandra Pasquevich[2], Stéphane Méresse[1], Juliana Cassataro[2], Sylvie Mémet[1‡] and Jean-Pierre Gorvel[1‡#]

1 Aix Marseille Univ, CNRS, INSERM, CIML, Centre d'Immunologie de Marseille-Luminy, Marseille, France
2 Instituto de Investigaciones Biotecnológicas Universidad Nacional de San Martín (UNSAM)-CONICET, Buenos Aires, Argentina

* Co-first authors

‡ Co-senior and corresponding authors: memet@ciml.univ-mrs.fr; gorvel@ciml.univ-mrs.fr

Lead contact: gorvel@ciml.univ-mrs.fr ; Tel: +33491269115

Running title: The Omp25/SLAMF1 axis in DCs favours *Brucella* chronicity

Keywords: *Brucella*, Omp25, SLAMF1, CD150, dendritic cells, NF-κB, inflammation, acute and chronic infection

Footnote:

Present addresses: C. Degos, CRCM - Immunomonitoring Platform/ Tumour Immunology, Marseille, France; A. Gagnaire, Faculty of Veterinary Medicine: Merelbeke, Belgium ; A. Papadopoulos, HalioDX, Marseille, France

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cmi.13164

Abstract

The strategies by which intracellular pathogenic bacteria manipulate innate immunity to establish chronicity are poorly understood. Here we show that *B. abortus* outer membrane protein Omp25 specifically binds the immune cell receptor SLAMF1 *in vitro*. The Omp25-dependent engagement of SLAMF1 by *B. abortus* limits NF-κB translocation in dendritic cells (DC) with no impact on *Brucella* intracellular trafficking and replication. This in turn decreases pro-inflammatory cytokine secretion and impairs DC activation. The Omp25-SLAMF1 axis also dampens the immune response without affecting bacterial replication *in vivo* during the acute phase of *Brucella* infection in a mouse model. In contrast, at the chronic stage of infection, the Omp25/SLAMF1 engagement is essential for *Brucella* persistence. Interaction of a specific bacterial protein with an immune cell receptor expressed on the DC surface at the acute stage of infection is thus a powerful mechanism to support microbe settling in its replicative niche and progression to chronicity.

Introduction

A key issue for intracellular bacteria is to find a way to disseminate and survive to establish chronic infection. Bacteria have therefore developed efficient strategies to evade immune system recognition and elimination (Brodsky and Medzhitov, 2009; Sansonetti and Di Santo, 2007). Targeting the signalling triggered by pattern recognition receptors in antigen presenting cells is one of them (Byndloss and Tsolis, 2016b). However little is known about other cell surface host-bacteria interacting factors that contribute to promoting chronicity and a firm demonstration of a direct link between such interaction and progress towards chronic infection is still under scrutiny.

*Brucell*a spp. are Gram-negative facultative intracellular coccobacilli, which are the causative agent of brucellosis, a worldwide re-emerging zoonosis affecting live stock (bovine for *B. abortus*, ovine and caprine for *B. melitensis*, swine for *B. suis*) and wild life with serious economic loss, as *Brucella* infected animals undergo spontaneous abortion and infertility (Corbel, 1997; Cutler et al., 2005; Moreno, 2014). Brucellosis can be transmitted to humans via contaminated food or infected aerosol particles. Acute human brucellosis is characterised by an undulant febrile illness, which if left untreated as often the case, leads to chronic inflammation (Dean et al., 2012; Pappas et al., 2005). Due to its high infectivity, *Brucella* has been classified as a potential warfare agent (Robinson-Dunn, 2002) and is manipulated in BSL3 only. *Brucella* spp. have the ability to enter, survive and replicate within non-phagocytic or phagocytic cells, and as such evade host immune system defence mechanisms (Byndloss and Tsolis, 2016a; Gorvel and Moreno, 2002; Martirosyan et al., 2011; von Bargen

et al., 2012). These properties may account for human brucellosis particularities (Skendros et al., 2011).

Dendritic cells (DCs), which display a higher susceptibility than macrophages to *Brucella* infection (Billard et al., 2005), are essential for the induction of innate and adaptive immune responses against this pathogen (Avila-Calderon et al., 2019; Papadopoulos and Gorvel, 2015). DCs control bacterial growth through secretion of pro-inflammatory cytokines such as interleukin-12 (IL-12) or enzyme like NOS2 (Copin et al., 2007; Macedo et al., 2008). A number of bacterial effectors, including BtpA and BtpB proteins (Salcedo et al., 2013; Salcedo et al., 2008), and a non-canonical LPS (Conde-Alvarez et al., 2012; Zhao et al., 2018), help *Brucella* survive in phagosomes, settle in their safe replication niche, the endoplasmic reticulum (ER), and/or modulate DC activation. The outer membrane protein Omp25, a major transmembrane protein, also regulates immune response against *Brucella*. *In vitro*, *Brucella* Omp25-defective mutants (Δ*omp25*) induce human monocyte (mo)-DC and macrophage activation, which results in TNF-α and IL-12 secretion (Billard et al., 2007; Jubier-Maurin et al., 2001). *In vivo*, the role of *Brucella* Omp25 is controversial since *B. abortus* or *B. melitensis* Δ*omp25* mutants are either attenuated (Edmonds et al., 2002) or as virulent as the wild-type parental strains (Manterola et al., 2007). Even though Omp25 has been considered as a potential vaccine (Byndloss and Tsolis, 2016a; Commander et al., 2010; Commander et al., 2007; Goel and Bhatnagar, 2012; Goel et al., 2013), further work is needed to clarify the role of this outer membrane protein in the regulation of host immune responses.

A previous transcriptomics study led us identify *SLAMF1* as a strongly upregulated gene in human monocyte-derived DCs (mo-DCs) stimulated with *Brucella* β1,2 cyclic glucan (CβG)

(Degos et al., 2015; Martirosyan et al., 2012). SLAMF1 is a cell surface receptor belonging to the signalling lymphocyte activation molecule family (SLAMF), itself a member of the Immunoglobulin (Ig) superfamily. SLAMF1 is expressed on hematopoietic cells only and constitutes a self-ligand that triggers T cell activation (Cannons et al., 2011; Fouquet et al., 2018). SLAMF1 is involved in immune responses against various pathogens (van Driel et al., 2016). Identified as the entry receptor for Measles virus, it controls DC and T cell responses along viral infection. In macrophages, SLAMF1 binds *S.* Typhimurium and *E. coli* outer membrane proteins OmpC and OmpF, thus regulating bacterial phagosome function and killing (Berger et al., 2010; Ma et al., 2012). SLAMF1 plays a dual role as both an activator and an inhibitor of the immune system during *M. tuberculosis* and *T. cruzi* infections (Calderon et al., 2012; Pasquinelli et al., 2004). Its role in brucellosis has not yet being investigated.

Here, we demonstrate a specific direct interaction of *Brucella* Omp25 with the SLAMF1 receptor *in vitro*. We show that the Omp25-dependent engagement of SLAMF1 by *B. abortus* functionally limits the pro-inflammatory DC response *in vitro* as well as splenic DC subset expansion and T cell activation during acute infection *in vivo*, without affecting bacterial replication. We further prove that this restriction is essential for bacterial settling during chronic infection. Together our results bring to light a yet unrecognised avoidance strategy employed by *Brucella* to prevent recognition and elimination by the immune system thus disseminating and establishing its replication niche. Moreover, we show for the first time that bacterial replication can be uncoupled from the inflammatory status of the host.

Results

Brucella replication and persistence in bone marrow-derived DCs (BMDCs) are independent of Omp25 and SLAMF1

In a previous transcriptomic study, we were intrigued by the strong up-regulation of *SLAMF1* gene expression in human mo-DCs treated with *B. abortus* cyclic β1,2-glucan (CβG) (Degos et al., 2015) (**Table S1**). Since SLAMF1 overexpression follows binding of *E. coli* outer membrane proteins OmpC and OmpF to macrophages (Berger et al., 2010; Yurchenko et al., 2018) and Omp25 modulates human mo-DC activation upon *Brucella* infection (Billard et al., 2007), we explored whether *B. abortus* with or without Omp25 controlled murine SLAMF1 protein levels in BMDCs *in vitro*. BMDCs were challenged for 24 h with *B. abortus* wild-type (Ba WT) or a Omp25-defective mutant *(*Ba Δ*omp25*) or its complemented mutant strain that stably re-expressed Omp25 (Ba Δ*omp25*pBBR4*omp25*) or mock-treated or treated with *E. coli* LPS or CβG, as reported (Martirosyan et al., 2012; Zhao et al., 2018) and phenotypic investigation was done by flow cytometry (**Figure 1a)**. A marked and similar increase in SLAMFI levels, stronger than that obtained in CβG- or *E. coli* LPS-treated cells, was observed with all types of infected samples compared to those of mock-treated BMDCs, indicative of a *Brucella* Omp25-independent control of SLAMF1 upregulation (**Figure 1b**). We then analysed the intracellular replication of the Ba Δ*omp25* mutant within BMDCs and compared it with that of Ba WT, Δ*omp25*pBBR4*omp25* or Δ*virB* strains. The Ba Δ*omp25* mutant replicated intracellularly in BMDCs at a rate comparable to that of WT or Δ*omp25*pBBR4*omp25* strains in contrast to a non-replicative strain, the *virB* mutant (**Figure 2a**), which persisted for 48 h only (Sieira et al., 2000). The Δ*omp25* strain replicated within

the ER similarly to the WT strain (**Figure 2b**), showing that both *Brucella* replication and intracellular trafficking in DC are Omp25-independent. When assessing *Brucella* replication in wild type (wt) or *Slamf1*$^{-/-}$ BMDCs (**Figure 2c)**, equivalent replication rates were obtained with all strains or DC genetic backgrounds, indicating that as for Omp25, SLAMF1 engagement does not affect *Brucella* replication.

BMDC activation is restrained in an Omp25/SLAMF1-dependent mode upon *Brucella* infection

We then characterized by flow cytometry the phenotype of BMDCs at 24 h p.i.. In Ba WT-infected BMDCs, DC activation, reflected by CD80, CD86, CD40 co-stimulatory molecule expression levels (**Figure 1a)**, was low compared to that of *E. coli* LPS-treated control cells (**Figure 3a**), as reported (Conde-Alvarez et al., 2012). However, Ba Δ*omp25*-infected BMDCs exhibited significantly higher expression levels, although still below those of *E. coli* LPS-treated cells. The rescue of the Δ*omp25* mutant phenotype by the complemented strain (Δ*omp25*pBBR4*omp25*) demonstrated a direct role of Omp25 in controlling DC surface marker expression. MHCII expression was stable whatever *Brucella* strain considered and below that obtained in LPS-treated cells (**Figure S1**), supporting the notion that the Ba Δ*Omp25* strain triggers an intermediate level of DC activation. In *Slamf1*$^{-/-}$ BMDCs, surface activation markers were expressed at similar levels regardless of the stimulus (LPS, WT or mutant bacteria) (**Figures 3a** and **S1a**). Collectively, this revealed a partial Omp25/SLAMF1-mediated decrease of BMDC activation upon *Brucella* infection. Likewise, Ba Δ*omp25* infected BMDCs displayed much higher levels of TNF-α, IFN-γ and IL-6 secretion than those

in mock or Ba WT-infected wt cells (**Figure 3b**). In *Slamf1*$^{-/-}$ BMDCs, cytokine secretion elicited by *Brucella* WT strain was equivalent to that of the Δ*omp25* strain (**Figure 3b**). The higher cytokine secretion of the Δ*omp25* mutant phenotype in wt BMDCs and loss of difference between the *Brucella* strains in the SLAMF1-deficient BMDCs demonstrated the Omp25/SLAMF1-mediated control of DC activation. Hence, the Omp25/SLAMF1 axis finely restricts the host inflammatory response triggered by *Brucella* infection in DCs.

Purified recombinant *Brucella* Omp25 protein binds SLAMF1 directly

To determine whether Omp25 is a ligand for the SLAMF1 receptor, we constructed a plasmid encoding the N-terminal Myc-tagged extracellular domain of SLAMF1. A similar construct with the extracellular domain of another member of the Ig receptor family, CD90, was used as a negative control. Immunoprecipitated Myc-containing complexes from protein extracts of transfected COS-7 cells were incubated with purified recombinant *Brucella* Omp25 and analysed by Western blot (**Figure 4a**). SLAMF1 extracellular domain pulled-down Omp25, in contrast to that of CD90, which was unable to do so in the presence of 0.1 ng of Omp25, although a faint interaction was detected with 1 ng of this recombinant protein. We inferred that *B. abortus* Omp25 protein interacted specifically with ectopically expressed SLAMF1 extracellular domain since it did not occur with another member of the super Ig family. To test if this interaction was shared by other *Brucella* Omps, the same pull-down approach was followed using 0.1 ng of Omp19 (**Figure 4b**). Purified recombinant *B. abortus* Omp19 protein did not interact with either SLAMF1 or CD90 extracellular domains. These findings demonstrate the specificity of the interaction of SLAMF1 with *Brucella* Omp25 protein.

The Omp25-dependent engagement of SLAMF1 by *Brucella* controls NF-κB nuclear translocation in BMDCs upon infection

To assess the impact of the Omp25-dependent engagement of the SLAMF1 receptor by *Brucella* at early stages of BMDC activation *in vitro*, we then monitored the translocation of NF-κB to the nucleus at 2 h p.i. by confocal immunofluorescence (**Figure 5**). BMDCs infected with Ba Δ*omp25* displayed a significantly higher level of nuclear NF-κB than cells infected with Ba WT. The rescue of the Δ*omp25* mutant phenotype by the complemented strain, Δ*omp25*pBBR4*omp25*, confirmed the role of Omp25 in limiting nuclear translocation of NF-κB in *Brucella*-infected BMDCs. A significant and comparable increase in nuclear NF-κB was observed in *Slamf1*$^{-/-}$ BMDCs following infection with Ba WT or Δ*omp25* (**Figures 5 and S2**) relative to levels in WT cells. A similar result was obtained in WT BMDCs in the presence of a SLAMF1 blocking peptide (**Figure S2**). The Omp25-dependent engagement of SLAMF1 by *Brucella* in DCs thus limits nuclear translocation of NF-κB, an essential feature for pro-inflammatory gene transcription. In accordance with enhanced nuclear NF-κB translocation, Δ*omp25* infection of BMDCs resulted in an over-expression of *Il6*, *Il12b*, *Ccl2*, *Il1b*, *Tnfa*, *Cxcl1*, *Nos2* and *Ptsg*2 at both 6 h and 24 h p.i. compared with cells infected with the Ba WT strain (**Figure S3**). Therefore, the Omp25-SLAMF1 axis restricts the DC inflammatory response during *Brucella* infection.

The Omp25-dependent engagement of SLAMF1 by *Brucella* limits acute inflammation without altering *Brucella* burden *in vivo*

To investigate the role of the Omp25-dependent engagement of SLAMF1 by *Brucella in vivo*, we carried out infection studies in C57BL/6 mice (Grillo et al., 2012). Spleens were first assessed 8 days after intraperitoneal challenge (**Figure 6a**) during the acute phase of infection. A significant increase in spleen weight of Δ*omp25*-infected wt mice or *Slamf1*$^{-/-}$ mice compared to that of Ba WT-infected C57BL/6J mice suggested an increased inflammatory response in the absence of Omp25 or SLAMF1, similar to our findings *in vitro*. Interestingly, equivalent bacterial counts in spleen were enumerated whatever *Brucella* strain or mice considered. No significant difference in granuloma formation, thought to represent the host's ability to develop a protective immune response, was detected between the Ba WT-infected mice and any other infected animals, although there was a trend for an increase in Ba Δ*omp25*- versus Ba WT-infected spleens (**Figure S4**). The Omp25-dependent SLAMF1 engagement by *Brucella* seemed to finely tune down the inflammatory response early on *in vivo* during infection without affecting bacterial replication itself. To test such a hypothesis, we used a lethal model of mouse brucellosis, the immuno-compromised *Ifng*$^{-/-}$ mice (Brandao et al., 2012). When infected with the Ba WT strain, these mutant mice survived significantly longer (until day 30) than those infected with the Δ*omp25* strain (all dead by day 25) (**Figure 6b**), despite equivalent bacterial CFU counts in the spleen at day 20 p.i. (median of 5.08 ± 0.3 logCFU/spleen for Ba WT versus 5.27± 0.37 logCFU/spleen for the Δ*omp25* mutant). As expected, infected animals in a wt background showed no effect on mouse viability. The reduced survival of Δ*omp25*-infected *Ifng*$^{-/-}$ mice most likely reflects a precocious hyper-inflammation triggered by the absence of Omp25. Altogether, the Omp25/SLAMF1 axis

restrained inflammation at early stages of *Brucella* infection *in vivo* without modifying the bacterial burden itself.

The Omp25-dependent engagement of SLAMF1 by *Brucella* prevents splenic expansion of all DC subsets, and inhibits downstream T cell activation during the acute phase of infection *in vivo*

The fact that spleens of wt mice infected by the Δ*omp25* mutant were significantly heavier than that of mice infected with Ba WT strain and similar to those of Ba WT- or Δ*omp25*-infected *Slamf1*$^{-/-}$ mice, raised the possibility of *in viv*o Omp25/SLAMF1-dependent changes in cellularity of immune cells upon infection. To determine if it was the case, DC splenic subsets identified as in **Figure 7a**, were analysed by flow cytometry at 8 days p.i. (**Figure 7b**). Absolute numbers of cDC1, cDC2 and pDC were slightly augmented upon infection with Ba WT or Δ*omp25*pBBR4*omp25* strains compared to those detected in Mock PBS-injected mice. Loss of Omp25 led to a further rise of these three DC subpopulations to levels akin to those seen upon infection with Ba WT or Δ*omp25* of *Slamf1*$^{-/-}$ mice. We next asked whether this *in vivo* modulation of DC splenic subpopulations by the Omp25-dependent engagement of SLAMF1 by *Brucella* affected downstream T cell activation. Percentages and absolute numbers of $CD4^+CD69^+$ T cells, examined as in **Figure 8a**, increased with the lack of Omp25 in *Brucella* or of SLAMF1 in the host (**Figures 8b and S5**). The Δ*omp25* mutant phenotype was again rescued by the complemented strain in wt-infected animals only. Absolute numbers of $CD8^+CD69^+$ T cells fluctuated similarly (**Figures 8b and S5**). The Omp25/SLAMF1 axis

thus directly controls the expansion of splenic DC subsets and activated T cells in acute infection.

The Omp25-dependent engagement of SLAMF1 facilitates persistence of *Brucella* at the chronic phase of infection *in vivo*

When finally exploring the impact of the Omp25-dependent engagement of SLAMF1 by *Brucella* in the chronic phase of mouse infection, the situation was different from the acute phase (**Figure 9**). Spleen weights of all infected mice were similar at day 30 p.i.; however, a significant reduction in bacterial loads was observed in the spleens of wt mice infected with the Ba Δ*omp25* mutant or *Slamf1*$^{-/-}$ mice infected with either Ba strains compared to that in wt mice infected with Ba WT. The Δ*omp25* mutant phenotype was rescued by the complemented strain Δ*omp25*pBBR4*omp25*, clearly demonstrating the requirement of Omp25 for optimal settling of *Brucella* at the chronic phase. The comparable phenotype of Δ*omp25* mutant in wt mice and of the Ba WT and mutant strains in *Slamf1*$^{-/-}$ mice further supported the assumption that Omp25 operates through the engagement of the SLAMF1 receptor.

Discussion

In this study, we identify a role for SLAMF1 in establishment of chronic *Brucella* infection and discover a previously unrecognized interaction partner, *B. abortus* Omp25, in addition to the known Measles virus, *S.* Typhimurium and *E. coli* OmpC and OmpF porins. We demonstrate that SLAMF1 specifically interacts with *B. abortus* Omp25 *in vitro*. Other members of the SLAM receptor family sense various microbial components like SLAMF2 that binds *E. coli* lectin FimH (van Driel et al., 2016). Future work will determine if other features of *Brucella* are recognized by other SLAM family member(s). Several *Brucella* Omp25 paralogs have been described. The Omp25 family is composed of Omp25/Omp25a, Omp25b, Omp25c and Omp25d. Expression of the genes coding for the Omp25 family in *Brucella* spp. have been assessed both at the transcriptomic and protein levels (Martin-Martin et al., 2009). Omp25b was not detected in *B. abortus* and Omp25d was not detected in any *Brucella* spp.. The only paralog beside Omp25/Omp25a that is expressed by *B. abortus* is Omp25c and nothing is known about its role in brucellosis. Further investigation will assess whether Omp25c interacts as Omp25 with SLAMF1.

Brucella Omp proteins have been proposed to be masked by the long-O-chain of LPS, notably from complement C1q binding (Eisenschenk et al., 1999). However, several independent sets of experiments have demonstrated the exposure and accessibility of Omp25 proteins at the surface of *Brucella* spp.. As such, immunoelectron microscopy using specific anti-Omp25 antibody detected Omp25 at the surface of B. *melitensis* B115 cells (Cloeckaert et al., 1996). Anti-Omp25 antibodies reversed the inhibition of TNF-α elicited by *B. suis* infection in a human macrophage line (Jubier-Maurin et al., 2001). Given the extremely high homology

between Omp25 proteins of *Brucella* spp., it is more than likely that the same holds true for the Omp25 protein of *B. abortus*. Moreover, the work of Jubier-Maurin et al. supports a direct role of Omp25 in the restriction of TNF-α synthesis and secretion seen in our BMDCs during *B. abortus* infection (Figures 3(b) and S3), which also requires SLAMF1, as shown by using SLAMF1-deficient cells or SLAMF1 blocking peptide (Figures 3(b) and S2). Variations in the apparent molecular sizes of Omp proteins have been attributed to a possible association with peptidoglycan of different sizes (Cloeckaert et al., 1992; Sowa et al., 1991). Our *in vitro* pull-down experiments with total protein extracts from Cos7 cells ectopically expressing SLAMF1 extracellular domain and put in contact with purified recombinant Omp25 protein prove that the interaction of Omp25 with SLAMF1 is direct and does not involve other bacterial component. For all these reasons, we consider that upon infection, *B. abortus* Omp25 is not only exposed but has the ability to make direct contact with the SLAMF1 receptor at the surface of dendritic cells, although this binding, shown *in vitro*, was not formally demonstrated *in vivo*. A possibility thus remains that alternatively, deletion of *omp25* deprives *Brucella* from a component that phenocopies SLAMF1 deficiency in DCs *in vivo*.

The Omp25-dependent engagement of SLAMF1 by *B. abortus* in DCs results in a different outcome from that triggered by *E. coli* OmpC and OmpF in macrophages (Berger et al., 2010; Ma et al., 2012). We show that neither loss of Omp25 nor SLAMF1 affected *Brucella* intracellular trafficking or bacterial replication. We confirmed our data *in vivo*, demonstrating in mice that *Brucella* replication is independent of Omp25 and SLAMF1 during acute infection. However, when Omp25 or SLAMF1 are missing, the bacterial burden is

significantly diminished during chronic infection. By itself the SLAMF1-Omp25 interaction does not directly control bacterial replication during early infection but alters the host's response in such way that as a secondary consequence it influences bacterial load at the chronic stage. SLAMF1 exhibits versatile functions resulting in either pro- or anti-inflammatory outcomes (Bleharski et al., 2001; Rethi et al., 2006). Here, the Omp25-dependent engagement of SLAMF1 by *Brucella* in DCs limited nuclear translocation of NF-κB, subsequently muting pro-inflammatory gene transcription, cytokine/chemokine secretion and co-stimulatory molecule expression *in vitro*. These findings are consistent with prior reports showing that *Brucella* growth in DCs is restrained through secretion of pro-inflammatory cytokines and that Δ*omp25* strains do not trigger bacterial attenuation in human mo-DCs or murine macrophages (Billard et al., 2007; Manterola et al., 2007). *In vivo*, the Omp25-dependent engagement of SLAMF1 by *Brucella* subtly down-regulated inflammation, without affecting bacterial burden during acute infection. This was exemplified by the earlier death of Ba Δ*omp25*-infected mice compared to WT-infected ones in the lethal model of brucellosis in spite of invariant bacterial loads. Decreased acute inflammation was illustrated in C57BL/6 infected mice by reduced cellularity of immune cells (less splenic DC subsets and activated T cells). We infer that the slight decrease of inflammation and activation of DCs and T cells at the acute phase of infection elicited by the Omp25-mediated engagement of SLAMF1, is not enough to affect bacterial replication per se, as evidenced by stable replication in DC *in vitro* and during acute infection *in vivo*. However, it may allow *Brucella* to better survive in its replicative niche in DCs, settle in and persist during the chronic phase of infection. During the acute phase of infection just after entry in the host, activated immune

cells are recruited in lymphoid organs. Later on, during chronic infection, infected cells change their metabolism and become anti-inflammatory (Byndloss and Tsolis, 2016b). When the Omp25/SLAMF1 axis is missing, immune cells might not switch their metabolism to an anti-inflammatory phenotype, and thus continue to produce inflammatory cytokines leading to the killing of infected cells. This in turn would result in decreased bacterial loads in *Brucella abortus* Δ*omp*25 strain-infected WT mice or in *Slamf1*$^{-/-}$ mice infected with *B. abortus* WT or Δ*omp*25 strains. Our data demonstrate that during acute infection bacterial replication can be uncoupled from the inflammatory status of the host and are consistent with the inhibitory effect of other *Brucella* effectors, BtpA and BtpB on the phenotypic maturation and function of DCs upon infection (Salcedo et al., 2008; Salcedo et al., 2013). BtpA and BtpB interact via their Toll interleukin-1 receptor (TIR) domain with the TLR signalling complex after being exported from bacterial membranes and translocated to the host cell cytosol (Salcedo et al., 2008;: Salcedo et al., 2013). However no significant differences were observed in bacterial CFU counts between WT *Brucella* and the btp mutants at different stages of infection (30, 60, 90, and 130 days) in BALB/c mice (Salcedo et al., 2013), known to exhibit a Th2 phenotype, supporting our metabolic switch hypothesis in C57BL/6 mice, which are more Th1. Further analyses will determine the validity of such hypothesis.

Our findings also suggest that the Omp25-dependent engagement of SLAMF1 by *Brucella in vivo* might target critical steps of DC-T cell interactions (Chudnovskiy et al., 2019), as SLAMF1 is recognized as a co-stimulatory molecule between DC and T cells (Cocks et al., 1995). Since without Omp25 *Brucella* was more potent in inducing expansion of all DC subsets and T cell activation, a possible mechanism might be that the Omp25-dependent

engagement of SLAMF1 by *B. abortus* impedes SLAMF1 homophilic interactions. SLAMF1 harbours in its cytoplasmic tail immunoreceptor tyrosine-based switch motifs (ITSM), used by phosphatases and kinases, recruited through the SLAM adaptor protein (SAP), to control T cell activation in a phospho-tyrosine dependent mode (Cannons et al., 2011; Detre et al., 2010). Recruitment of this cascade may alternatively be impaired. As SLAMF1 is essential for TLR4-mediated TRAM-TRIF-dependent signalling in *E. coli*-infected human macrophages (Yurchenko et al., 2018), another possibility is that its engagement by Omp25 affects the recruitment of other signalling receptor(s) or adapter protein(s). Further investigations will decipher how the engagement of the SLAMF1 receptor by *Brucella* Omp25 controls NF-κB nuclear translocation in DCs and define the transduction pathways involved and their interplay.

In conclusion, the Omp25-dependent engagement of SLAMF1 by *Brucella* illustrates a discrete evasion strategy exploited by a Gram-negative bacterium within infected DCs to mediate its dissemination *in vivo* by subtly controlling immune responses in a timely manner to foster chronicity. Immune modulation can thus be uncoupled from bacterial replication during acute infection. *Brucella* targeting SLAMF1 may have broader consequences given that, on the one hand, *Brucella* colonizing and persisting in murine bone marrow (Gutierrez-Jimenez et al., 2018) is transmitted by bone marrow transplantation in humans (Naparstek et al., 1982; Tuon et al., 2017), and on the other hand, SLAMF1 is a key cell surface receptor of hematopoietic stem cells (Oguro et al., 2013). Investigating the impact of the Omp25-dependent engagement of SLAMF1 on hematopoietic stem cell maintenance and differentiation along *Brucella* infection should open promising research avenues.

Experimental procedures

Ethics

Animal experimentation was conducted in strict compliance with good animal practice as defined by the French animal welfare bodies (Law 87–848 dated 19 October 1987 modified by Decree 2001-464 and Decree 2001-131 relative to European Convention, EEC Directive 86/609). INSERM guidelines have been followed regarding animal experimentation (authorization No. 02875 for mouse experimentation). All animal work was approved by the Direction Départementale Des Services Vétérinaires des Bouches du Rhône and the Regional Ethic Committee (authorization number 13.118). Authorisation of *Brucella* experimentation in BSL3 facility was given under the numbers: AMO-076712016-5, AMO-076712016-6 and AMO-076712016-7. All efforts were made to minimize suffering during animal handling and experimentation.

Mice

6-10 week-old female C57BL/6J mice from Charles River, *Slamf1*$^{-/-}$ mice (kindly provided by Yusuke Yanagi) (Davidson et al., 2004) or *Ifng*$^{-/-}$ (Brandao et al., 2012) mice, both on a C57BL/6J background, were used. Animals were housed in cages with water and food ad libitum in the CIPHE animal house facility, Marseille. Two weeks before the start of experiments, mice were transferred to the BSL3, CIPHE, Marseille, and kept under strict biosafety containment conditions all along infection with live bacteria. Mice were inoculated intraperitoneally with $1x10^6$ CFU for each *Brucella* strain. For survival experiments, mice were weighed every two days; when reaching a weight loss of 30%, they were sacrificed.

Bacterial strains

B. abortus smooth virulent strain 2308 and the derived strain devoid of Omp25, Δ*omp25*, (corresponding to a deletion of the *omp25a* (BAB1_0722) gene, which is regulated by BvrR/BvrS (Viadas et al., 2010)) have been described (Manterola et al., 2007). Details of the construction of the complemented version of *B. abortus* Δ*omp25* strain, Δ*omp25*pBBR4*omp25*, are presented below. *Brucella* strains were grown on Tryptone Soya Agar (TSA) plates, supplemented with kanamycin 25 μg/mL for the Δ*omp25* strain or kanamycin and ampicillin 50 μg/mL for the Δ*omp25*pBBR4*omp25* strain. For infection, strains were grown for 16 h approximately at 37°C under shaking in Tryptic Soy Broth (TSB), in presence of the appropriate antibiotics when required, until the OD at 600nm reached 1.8. All *Brucella* were kept, grown and used under strict biosafety containment conditions all along experiments in the BSL3 facility, Marseille. For sub-cloning of SLAMF1 and CD90 constructs, Top10 thermo-competent *E. coli* bacteria were used. Liquid cultures were incubated for 16h in Luria Broth (LB) at 37°C under constant shaking and in presence of the adequate antibiotics. Solid cultures were grown onto LB agar.

Construction of the complemented *B. abortus* Δ*omp25c*pBBR4*omp25* strain

The *B. abortus omp25a* gene was amplified by PCR using HiFi Taq polymerase according to manufacturer's instructions with primers described in **Table S2**. 7 μl of the PCR product were used for A tailing in presence of 1xTaq Buffer, 0.2 mM of ATP, 5 units of Taq during 30 min at 70°C. 3 μl of this reaction were then ligated into the pGEM-T vector, and ligation

product was used to transform *E. coli* JM109. Clones were purified by miniprep DNA kit (Promega) and checked by sequencing. 1 μg of pGEM-T-omp25 plasmid was digested with EcoRI HF and BamHI following manufacturer's instructions along with 1 μg of the pBBR-MCS4 (ampicillin-resistant destination vector). After separation by electrophoresis, DNA fragments were purified from agarose gel using Qiaquick Gel Extraction kit (Qiagen). 7.5 μl of insert (pGEM-T-omp25) and 2.5 μl of pBBR-MCS4 were used for ligation with the T4 DNA ligase for 16 h at 16°C. 5 μl of the ligation was then transformed in *E.coli* DH5α. The plasmid first purified by miniprep DNA kit (Promega) was then conjugated in *E. coli*. Briefly, 50 μl of E. coli S17 λpir containing pBBR-MCS4-omp25 were added to 1ml of a stationary culture of *B. abortus* Δ*omp25*. After 2 washes in TSB, the pellet was resuspended in 100 μl of TSB and plated for 4h at 37°C in TSA. Bacteria were then re-isolated in a plate containing ampicillin (50 μg/ml), kanamycin (25 μg/ml) and nalidixic acid (5 μg/ml) allowing the isolation of the *B. abortus* Δ*omp25*pBBR4*omp25* strain.

Cell Culture

BMDC were prepared from 6-8 week-old C57BL/6J or *Slamf1*$^{-/-}$ female femurs and tibias as previously described (Papadopoulos et al., 2016). Briefly, bone ends were cut off and bone marrow was flushed with RPMI 1640 supplemented with 5% FBS, and 50 μM 2-mercaptoethanol. Red blood cells were removed by 1 min exposure to 1xRBC lysis buffer solution. 3x10^6 cells were seeded onto 6-well plates in 5 mL medium containing 0.8 % supernatant of the J558L GM-CSF producing cell line. Medium was changed at day 2.5 and BMDCs were ready to use at day 5. Cells were mock-treated or stimulated with *E. coli* LPS

(100 ng/mL), *B. abortus* CβG (10 μg/mL) or infected for 16 h or 24h. Cells were collected for flow cytometry and supernatant was kept at -80°C for cytokine dosage.

Cell culture infection

BMDCs were infected at a multiplicity of infection (M.O.I.) of 30:1. Bacteria were centrifuged onto cells at 400 g for 10 min at 4 ºC and then incubated for 30 min at 37 ºC with 5% CO_2. Cells were washed twice with medium and incubated for 1 h in medium containing 100 μg/ml gentamicin to kill extracellular bacteria. Thereafter, antibiotics concentration was decreased to 20 μg/ml. To monitor bacterial intracellular survival, infected cells were washed 3 times in 1xPBS and lysed with 0.1% Triton X-100 in H_2O. Serial dilutions were plated in triplicates onto TSB agar to enumerate CFUs after 3 days at 37°C.

Construction of Myc-SLAMF1(exon 2-3) and Myc-CD90(exon 2-3):

cDNA of the mouse *Slamf1* gene was obtained from Origene and cDNA of the mouse *Thy1* gene was a kind gift from Claude Grégoire (CIML). The two first coding exons of each of these cDNA, which encoded the extracellular domain of SLAMF1 or CD90/Thy-1, were amplified by PCR using the primers detailed in **Table S2** and cloned into a pCMV-Myc vector using the Gateway Technology. Plasmids were transformed into thermo-competent *E. coli* Top10 for amplification and then purified with endotoxin-free MaxiPrep Plasmid Kit. Clones were checked by sequencing.

Expression and purification of Myc-SLAMF1 exon2-3 or Myc-CD90 exon 2-3:

10 μg of Myc-SLAMF1 or Myc-CD90 expression vectors were transfected into COS-7 cells using Fugene 6 (Promega) according to manufacturer's instructions. 48 h after transfection, cells were harvested and lysed into 1xPBS, 1 % Triton X-100 in presence of a protease inhibitor cocktail. Expression of the transfected proteins was confirmed by western blot against the Myc epitope.

Expression of recombinant Omp25 and Omp19 *Brucella* proteins

Recombinant U-Omp19 was produced as previously described (Pasquevich et al., 2009) and finally stored in following buffer: 50 mM NaH2PO4, 300 mM NaCl pH 7.4. To produce Omp25, the complete sequence of *B. abortus omp25* gene (GenBank_X79284.1) (de Wergifosse et al., 1995) was synthetized and subcloned into pET22(b)+ (Novagen) in frame with 6×His-tag (Genscript). Expression and purification was performed as reported (Goel and Bhatnagar, 2012). Recombinant purified Omp25 was finally refolded and stored at -20 ºC in refolding buffer (50 mM TrisHCl, 1 M NaCl, 0.2 mM DTT, 0.1 mM EDTA, 0.5 M L-Arginine-HCl, 10% Glycerol, 0.15 M Urea, 3.6 mM Imidazole).

Immunoprecipitation of Myc-SLAMF1 and Myc-CD90

50 μl of protein G-Dynabeads (Thermofisher) were coupled to 1 μg of anti-c-Myc antibody (9E10) during 1 h at 4°C and then incubated with whole transfected COS-7 cell extracts in 1xPBS, 0.1 % Triton X-100 and protease inhibitors for 1 h at 4°C. After washes in 1xPBS, 0.1 % NP-40, 0.1 % FBS and protease inhibitor cocktail, Myc-SLAMF1 and Myc-CD90 immunoprecipitated complexes were incubated 1 h with 1 ng or 0.1 ng of purified

recombinant *B. abortus* Omp25 or Omp19 at 4°C. Washes in 1xPBS, 0.5 M NaCl, 0.001 % SDS were then performed to eliminate non-specific interactions. Samples were heated at 70°C in Laemmli buffer and centrifuged for 10 min at 14,000 g. Supernatants were denatured at 95°C for 5 min, prior to loading on 12% SDS PAGE. Western blot against Omp25 or Omp19 was then performed using Mouse IgG True Blot HRP (Rockland) as a secondary antibody to avoid unspecific Ig binding.

RNA extraction and RT

Total RNA were extracted from infected BMDC using RNeasy Mini Kit (Qiagen) following the manufacturer's instructions. cDNAs were generated with 300 ng of RNA as a template using Quantitech Reverse Transcription Kit (Qiagen) according to manufacturer's recommendations.

qPCR

2 µl of cDNA corresponding to 6 ng of starting total RNA were used for qPCR. Amplification reactions were performed in duplicates with SYBR Green (Takara) in 7500 Fast Real-time PCR (Applied Biosystem). *Hprt* was used as a housekeeping gene to normalize expression among samples and determine ΔCt. Data are presented as fold increase versus Mock treated cells, put arbitrarily at 1.

Cytokine dosage

Cytokine profiles (TNFα, IFNγ, and IL-6) in BMDC culture supernatants were analysed by cytometric beads assay (BD Biosciences, Mouse Inflammation kit).

Flow cytometry

Cells were stained for 20 min at 4°C with the antibodies listed in **Table S2**, then washed once in 2% FBS in 1xPBS and once in 1xPBS. Infected cells were then fixed for 20 min in 3 % PFA at 22°C. Events were collected by flow cytometry using a FACS LSRII UV or Fortessa (BD Biosciences) and analysis was performed on FACS DIVA and FlowJo softwares. For BMDC experiments, at least 100,000 $CD11c^{+}$ (DC marker) cells were analysed for MFI measurements. For *in vivo* experiments, DC subset identification followed the following strategy: after exclusion of neutrophils, NK cells, B cells, and T cells from live singlet cells, $CD11c^{pos}$ cells were divided into $CD11c^{int}CD11b^{-}Bst2^{+}SiglecH^{+}$ plasmacytoid DC (pDC) and $CD11c^{hi}$ cells, further identified as $XCR1^{+}SIRP\alpha^{-}$ conventional DC type (cDC)1 and $XCR1^{-}SIRP\alpha^{+}CD24^{-}$ cDC2 cells as described (Mattiuz et al., 2018; Schlitzer et al., 2015). T cells were identified from live singlet splenic cells by CD3 positivity. $CD4^{+}$ and $CD8^{+}$ T cell subsets were then assessed for expression of the activation marker CD69.

Immunofluorescence microscopy

Cells were fixed in 3.2 % paraformaldehyde, pH 7.4, at 22°C for 20 min. For NF-κB localisation studies, cells were then permeabilized for 10 min with 0.1 % saponin in 1xPBS, followed by 1 h blocking with 2 % BSA in PBS. Primary antibodies were incubated for 1 h followed by 2 washes in 1xPBS, 45 min incubation with secondary antibodies, 2 washes in

1xPBS and 1 wash in water before mounting with Prolong Gold (ThermoFisher). For the other immunofluorescence labelling, 2% BSA in 1xPBS was used for 1 h to block non-specific interactions. Then primary antibodies were incubated for 30 min in 1xPBS, 0.1 % saponin, 0.1 % horse serum. Coverslips with cells were then washed twice in 1xPBS, 0.1 % saponin before 30 min incubation with secondary antibodies and were finally mounted in Prolong Gold. Samples were examined on a Leica SP5 laser scanning confocal microscope for image acquisition. Images of 1024x1024 pixels were then assembled using Adobe Photoshop or ImageJ. In all experiments, an anti-CD11c antibody was used to restrict analysis to DC only. Antibodies are listed in **Table S2**. Quantifications were achieved by counting at least 50 DCs from 5 independent experiments, for a total of at least 250 DC analysed.

Mouse infection

6-8 week-old female C57BL/6J (wild-type), *Slamf1*$^{-/-}$ or *Ifng*$^{-/-}$ mice were infected in the BSL3 facility by intraperitoneal (IP) injection. 1x10^6 CFU were injected into 200 µl of sterile endotoxin-free PBS for each mouse. Organs were harvested at 8, 20 or 30 days p.i, weighted and then dissociated into sterile 0.1 % Triton X-100 diluted in H_2O. Serial dilutions in sterile 1xPBS were used to count CFU. Serial dilutions were plated in triplicates onto TSB agar to enumerate CFUs after 3 days at 37°C. For cytometry analyses, spleens were harvested, digested for 20 min at 37°C with type II collagenase and DNase I and then treated with 10 mM EDTA to stop digestion. Cut pieces of spleen crushed with a syringe plunger in a 70-µm nylon strainer cell strainer were filtered in 1xPBS, 5 mM EDTA, 2 % FBS. After removal of red cells by the Red blood cell lysis buffer (eBioscience) single splenic cell suspensions were

proceeded for FACS analysis. For histology studies, organs were harvested and placed into 10 % formalin for 24 h at 22°C before inclusion in paraffin. Slides were then stained with hematoxylin and eosin.

Statistical analysis

Statistical analyses were done using with the GraphPad Prism software. Brown-Forsythe ANOVA test followed by variance analysis with the Welch's test were performed, except for TNF-α dosage, for which the multiple comparison Kruskal Wallis ANOVA test, followed by variance analysis with the Dunn's test were applied. Mantel Cox test was used for survival curve. All values are expressed as mean ± standard deviation. Differences between values were considered significant at $p<0.05$. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$). All experiments were performed at least three times in triplicate otherwise indicated.

Acknowledgments

We are especially grateful to Y. Yanagi (Kyushu University, Japan) for providing us with the *Slamf1*$^{-/-}$ mice. We also thank I. López-Goñi (Universidad de Navarra, Pamplona, Spain) for his kind gift of the Omp25-deficent *B. abortus* strain. We are also thankful to X. De Bolle (Namur University, Belgium) for the *E. coli* S17 λpir strain, C. Grégoire (CIML) for the CD90 cDNA, A. Cloeckaert (INRA, Tours, France) for the rabbit anti-Omp25 and -Omp19 antibodies, L. Chasson and C. Laprie (CIML histology facility) for their help. We acknowledge the CIML PICSL imaging facility (ImagImm, member of the national infrastructure France-BioImaging), the CIML cytometry facility, and M. Sieweke and I. Moryión for helpful discussions. This work was supported by the French National Research Agency through the "Investments for the Future" program (France-BioImaging, ANR-10-INBS-04-01) and the “Laboratories of excellence” program (Labex INFORM, ANR-11-LABX-0054), the Fondation pour la Recherche Médicale (grant FRM-CS), the Excellence Initiative of Aix-Marseille University (AMU) - A*MIDEX, a French “Investissements d’Avenir” programme, and institutional grants from the CNRS, INSERM and AMU to the CIML.

Author contributions

Conceptualisation, C.D., L.H., V.A.G., Sy.M. and J.P.G.; Methodology, C.D., L.H., V.A.G., S.M., Sy.M. and J.P.G.; Investigation, C.D., L.H., V.A.G., G.G.E., A.G. and A.P.; Writing – Original Draft, Sy.M.; Writing – Review & Editing, C.D., L.H.,V.A.G., S.M., J.C., Sy.M. and

J.P.G.; Funding Acquisition, J.P.G.; Resources, R.C.A., K.A.P., and J.C..; Supervision, Sy.M. and J.P.G.

Declaration of Interests

The authors declare no competing interests.

Legends to Figures

Figure 1: In *B. abortus*-infected DCs, SLAMF1 overexpression is Omp25-independent. (a) Flow cytometry characterisation of DCs *in vitro* upon *B. abortus* infection. Representative FACS profiles of C57BL/6J BMDCs 24 h after mock-treatment or infection with wild-type *B. abortus* (Ba WT). Live singlet DCs were separated from macrophages ($F4/80^{+}$) and identified with the CD11c and MHCII cell surface markers. Expression levels of SLAMF1 and core DC activation markers (modal fluorescence) are shown. (b) SLAMF1 expression levels (MFI, Median of Fluorescence Intensity) measured by flow cytometry in DCs from C57BL/6J BMDCs 24 h after mock-treatment, stimulation with *E. coli* LPS or *B. abortus* CβG, or infection with Ba WT or Ba Δ*omp25* or Δ*omp25*pBBR4*omp25* mutant strains. 3-5 points per group, n= 3. Mean ± SD from pooled data. Significant differences from Ba WT-infected DCs. **, $p < 0.01$; ***, $p<0.001$. ns, non-significant. Brown-Forsythe ANOVA with Welch's correction.

Figure 2: In infected DCs, *B. abortus* replication is independent of both Omp25 and SLAMF1. (a) Kinetics of bacterial loads for BMDCs infected with *B. abortus* wild-type (Ba WT), Δ*omp25*, Δ*omp25*pBBR4*omp25* or Δ*virB* strains. Mean ± SD is shown, n=4. (b) Representative confocal microscopy images of C57BL/6J BMDCs infected with Ba WT or Δ*omp25* strains, fixed at 24 h p.i and stained for calnexin (ER staining, red) and anti-*Brucella* LPS (green). Scale bar 10 μm. 50 cells per group, n= 4. (c) Kinetics of bacterial loads for wt or *Slamf1*$^{-/-}$ BMDCs infected with Ba WT, Δ*omp25* or Δ*omp25*pBBR4*omp25* strains. Mean ± SD is shown, n=4. Mean ± SD from pooled data. Significant differences from Ba WT-infected

DCs. ***, $p<0.001$. Absence of *p* value, non-significant. Brown-Forsythe ANOVA with Welch's correction.

Figure 3: *B. abortus* infection inhibits DC activation in an Omp25-SLAMF1-dependent mode. (a) DC co-stimulatory molecule levels of expression (mean of fluorescence intensity, MFI) from wt or *Slamf1*$^{-/-}$ BMDCs mock-treated, stimulated with *E. coli* LPS or infected with Ba WT, Δ*omp25* or Δ*omp25*pBBR4*omp25* strains for 24 h. Data are expressed as MFI fold change versus MFI levels of one set of mock-treated cells put arbitrarily at 1. n=4. (b) Cytokine secretion levels from wt or *Slamf1*$^{-/-}$ BMDCs mock-treated or infected with Ba WT or Δ*omp25* for 24 h. n=3. Mean ± SD from pooled data. Significant differences from Ba WT-infected DCs. *, $p< 0.05$; **, $p < 0.01$; ***, $p<0.001$. Absence of *p* value or ns, non-significant. Brown-Forsythe ANOVA with Welch's correction.

Figure 4: *B. abortus* Omp25 specifically interacts with SLAMF1. Immunoblots of protein extracts from COS-7 cells expressing Myc-SLAMF1(exons 2-3) or Myc-CD90(exons 2-3) showing protein input and Omp25 binding (a) or Omp19 binding (b) to Myc-tag pull-down.

Figure 5: The Omp25-dependent engagement of SLAMF1 by *Brucella* limits NF-κB translocation in infected DCs**.** Confocal micrographs of wt or *Slamf1*$^{-/-}$ BMDCs mock-infected or infected with Ba WT, Δ*omp25* or Δ*omp25*pBBR4*omp25* for 2h, immunolabelled with TOPRO-3 (nucleus, yellow), anti-p65 (NF-κB, red) and anti-CD11c (DC, cyan). Scale bar, 10 μm. Bottom right, quantification of nuclear NF-κB in infected cells (n=4, at least 50

cells per group). Mean ± SD from pooled data. Significant differences from wt DCs infected with Ba WT. *, $p< 0.05$; ***, $p<0.001$. Absence of *p* value, non-significant. Brown-Forsythe ANOVA with Welch's correction.

Figure 6: The Omp25-dependent engagement of SLAMF1 by *Brucella* restricts inflammation without affecting bacterial replication during acute infection *in vivo*. (a) Spleen weights and bacterial loads for C57BL/6J wt and *Slamf1*$^{-/-}$ mice 8 days after intraperitoneal injection with PBS (Mock), *B. abortus* (Ba) WT or Δ*omp25* or Δ*omp25*pBBR4*omp25* strains. 3-5 mice per group, n=8. Significant differences from wt mice infected with Ba WT are shown. *, $p< 0.05$; **, $p< 0.01$; ***, $p<0.001$. Absence of *p* value or ns, non-significant. Brown-Forsythe ANOVA with Welch's correction. (b) Survival curve of wt (—) or *Ifng*$^{-/-}$ (—) mice infected with Ba WT (plain lines) or Δ*omp25* (dashed lines) strains. 5 mice per group, n=3. Mean ± SD from pooled data. Significant differences from mice infected with Ba WT are shown. *, $p< 0.05$. Absence of *p* value, non-significant. Mantel Cox test.

Figure 7: The Omp25-dependent engagement of SLAMF1 by *Brucella* restrains expansion of splenic DC subsets during acute infection *in vivo*. (a) Flow cytometry characterisation of splenic DCs *in vivo* upon *B. abortus* infection. Representative FACS profiles of one infected sample. For DC analysis, T cells, B cells, NK cells and neutrophils were first excluded from live singlet cells and remaining cells were analysed for CD11c and CD11b expression. Amongst the CD11ci^{int}CD11b^{-} cells, pDC were identified as Bst2^{+}SiglecH^{+} cells. The two conventional DC subsets were characterized among the CD11c^{hi} cells, as cDC1 cells for the

$XCR1^{+}SIRP\alpha^{-}$ fraction and as cDC2 cells for the $SIRP\alpha^{+}XCR1^{-}CD24^{-}$ fraction. (b) Absolute number fold changes of cDC1, cDC2 and pDC over mock-treated wild-type corresponding cells from wt or *Slamf1*$^{-/-}$ mice 8 days after intraperitoneal injection with PBS (Mock), *B. abortus* (Ba) WT or Δ*omp25* or Δ*omp25*pBBR4*omp25* strains. 3-5 mice per group, n=3. Mean ± SD from pooled data. Significant differences from wt mice infected with Ba WT are shown. *, $p< 0.05$; **, $p< 0.01$; ***, $p< 0.001$. Absence of *p* value, non-significant. Brown-Forsythe ANOVA with Welch's correction.

Figure 8: The Omp25-dependent engagement of SLAMF1 by *Brucella* inhibits T cell activation during acute infection *in vivo.* (a) Flow cytometry characterisation of splenic T cells *in vivo* upon *B. abortus* infection. Representative FACS profiles of one infected sample. T cells were identified from live singlet splenic cells by CD3 positivity. $CD4^{+}$ and $CD8^{+}$ T cell subsets were then assessed for expression of the activation marker CD69. (b) Absolute number fold changes of $CD4^{+}CD69^{+}$ and $CD8^{+}CD69^{+}$ T cells over mock-treated wild-type cells from wt or *Slamf1*$^{-/-}$ mice 8 days intraperitoneal injection with PBS (Mock), *B. abortus* (Ba) WT or Δ*omp25* or Δ*omp25*pBBR4*omp25* strains. 5-7 mice per group, n=2. Mean ± SD from pooled data. Significant differences from wt mice infected with Ba WT are shown. *, $p< 0.05$; **, $p< 0.01$. Absence of *p* value, non-significant. Brown-Forsythe ANOVA with Welch's correction.

Figure 9: The Omp25-dependent engagement of SLAMF1 by *Brucella* promotes bacterial persistence during chronic infection *in vivo*. Spleen weights and bacterial loads for wt and

Slamf1$^{-/-}$ mice 30 days after injection with PBS (Mock) or *B. abortus* (Ba) WT or Δ*omp25* or Δ*omp25*pBBR4*omp25* strains. 3-5 mice per group, n=5. Mean ± SD from pooled data. Significant differences from wt mice infected with Ba WT are shown. *, $p < 0.05$; ***, $p < 0.001$. Absence of *p* value or ns, non-significant. Brown-Forsythe ANOVA with Welch's correction.

Bibliography

Avila-Calderon, E.D., Flores-Romo, L., Sharon, W., Donis-Maturano, L., Becerril-Garcia, M.A., Arreola, M.G.A., Reynoso, B.A., Guemes, F.S., and Contreras-Rodriguez, A. (2019). Dendritic cells and Brucella spp. interaction: the sentinel host and the stealthy pathogen. Folia Microbiol (Praha).

Berger, S.B., Romero, X., Ma, C., Wang, G., Faubion, W.A., Liao, G., Compeer, E., Keszei, M., Rameh, L., Wang, N.*, et al.* (2010). SLAM is a microbial sensor that regulates bacterial phagosome functions in macrophages. Nat Immunol *11*, 920-927.

Billard, E., Cazevieille, C., Dornand, J., and Gross, A. (2005). High susceptibility of human dendritic cells to invasion by the intracellular pathogens Brucella suis, B. abortus, and B. melitensis. Infect Immun *73*, 8418-8424.

Billard, E., Dornand, J., and Gross, A. (2007). Brucella suis prevents human dendritic cell maturation and antigen presentation through regulation of tumor necrosis factor alpha secretion. Infection and Immunity *75*, 4980-4989.

Bleharski, J.R., Niazi, K.R., Sieling, P.A., Cheng, G., and Modlin, R.L. (2001). Signaling lymphocytic activation molecule is expressed on CD40 ligand-activated dendritic cells and directly augments production of inflammatory cytokines. J Immunol *167*, 3174-3181.

Brandao, A.P., Oliveira, F.S., Carvalho, N.B., Vieira, L.Q., Azevedo, V., Macedo, G.C., and Oliveira, S.C. (2012). Host susceptibility to Brucella abortus infection is more pronounced in IFN-gamma knockout than IL-12/beta2-microglobulin double-deficient mice. Clin Dev Immunol *2012*, 589494.

Brodsky, I.E., and Medzhitov, R. (2009). Targeting of immune signalling networks by bacterial pathogens. Nat Cell Biol *11*, 521-526.

Byndloss, M.X., and Tsolis, R.M. (2016a). Brucella spp. Virulence Factors and Immunity. Annu Rev Anim Biosci *4*, 111-127.

Byndloss, M.X., and Tsolis, R.M. (2016b). Chronic Bacterial Pathogens: Mechanisms of Persistence. Microbiol Spectr *4*.

Calderon, J., Maganto-Garcia, E., Punzon, C., Carrion, J., Terhorst, C., and Fresno, M. (2012). The receptor Slamf1 on the surface of myeloid lineage cells controls susceptibility to infection by Trypanosoma cruzi. PLoS Pathog *8*, e1002799.

Cannons, J.L., Tangye, S.G., and Schwartzberg, P.L. (2011). SLAM family receptors and SAP adaptors in immunity. Annu Rev Immunol *29*, 665-705.

Chudnovskiy, A., Pasqual, G., and Victora, G.D. (2019). Studying interactions between dendritic cells and T cells in vivo. Curr Opin Immunol *58*, 24-30.

Cloeckaert, A., Verger, J.M., Grayon, M., Zygmunt, M.S., and Grepinet, O. (1996). Nucleotide sequence and expression of the gene encoding the major 25-kilodalton outer membrane protein of Brucella ovis: Evidence for antigenic shift, compared with other Brucella species, due to a deletion in the gene. Infect Immun *64*, 2047-2055.

Cloeckaert, A., Zygmunt, M.S., de Wergifosse, P., Dubray, G., and Limet, J.N. (1992). Demonstration of peptidoglycan-associated Brucella outer-membrane proteins by use of monoclonal antibodies. J Gen Microbiol *138*, 1543-1550.

Cocks, B.G., Chang, C.C., Carballido, J.M., Yssel, H., de Vries, J.E., and Aversa, G. (1995). A novel receptor involved in T-cell activation. Nature *376*, 260-263.
Commander, N.J., Brewer, J.M., Wren, B.W., Spencer, S.A., Macmillan, A.P., and Stack, J.A. (2010). Liposomal delivery of p-ialB and p-omp25 DNA vaccines improves immunogenicity but fails to provide full protection against B. melitensis challenge. Genet Vaccines Ther *8*, 5.
Commander, N.J., Spencer, S.A., Wren, B.W., and MacMillan, A.P. (2007). The identification of two protective DNA vaccines from a panel of five plasmid constructs encoding Brucella melitensis 16M genes. Vaccine *25*, 43-54.
Conde-Alvarez, R., Arce-Gorvel, V., Iriarte, M., Mancek-Keber, M., Barquero-Calvo, E., Palacios-Chaves, L., Chacon-Diaz, C., Chaves-Olarte, E., Martirosyan, A., von Bargen, K.*, et al.* (2012). The Lipopolysaccharide Core of Brucella abortus Acts as a Shield Against Innate Immunity Recognition. Plos Pathogens *8*.
Copin, R., De Baetselier, P., Carlier, Y., Letesson, J.J., and Muraille, E. (2007). MyD88-Dependent Activation of B220-CD11b+LY-6C+ Dendritic Cells during Brucella melitensis Infection. The Journal of Immunology *178*, 5182-5191.
Corbel, M.J. (1997). Brucellosis: an overview. Emerg Infect Dis *3*, 213-221.
Cutler, S.J., Whatmore, A.M., and Commander, N.J. (2005). Brucellosis--new aspects of an old disease. J Appl Microbiol *98*, 1270-1281.
Davidson, D., Shi, X., Zhang, S., Wang, H., Nemer, M., Ono, N., Ohno, S., Yanagi, Y., and Veillette, A. (2004). Genetic evidence linking SAP, the X-linked lymphoproliferative gene product, to Src-related kinase FynT in T(H)2 cytokine regulation. Immunity *21*, 707-717.
de Wergifosse, P., Lintermans, P., Limet, J.N., and Cloeckaert, A. (1995). Cloning and nucleotide sequence of the gene coding for the major 25-kilodalton outer membrane protein of Brucella abortus. J Bacteriol *177*, 1911-1914.
Dean, A.S., Crump, L., Greter, H., Hattendorf, J., Schelling, E., and Zinsstag, J. (2012). Clinical manifestations of human brucellosis: a systematic review and meta-analysis. PLoS Negl Trop Dis *6*, e1929.
Degos, C., Gagnaire, A., Banchereau, R., Moriyon, I., and Gorvel, J.P. (2015). Brucella CbetaG induces a dual pro- and anti-inflammatory response leading to a transient neutrophil recruitment. Virulence *6*, 19-28.
Detre, C., Keszei, M., Romero, X., Tsokos, G.C., and Terhorst, C. (2010). SLAM family receptors and the SLAM-associated protein (SAP) modulate T cell functions. Semin Immunopathol *32*, 157-171.
Edmonds, M.D., Cloeckaert, A., and Elzer, P.H. (2002). Brucella species lacking the major outer membrane protein Omp25 are attenuated in mice and protect against Brucella melitensis and Brucella ovis. Vet Microbiol *88*, 205-221.
Eisenschenk, F.C., Houle, J.J., and Hoffmann, E.M. (1999). Mechanism of serum resistance among Brucella abortus isolates. Vet Microbiol *68*, 235-244.
Fouquet, G., Marcq, I., Debuysscher, V., Bayry, J., Rabbind Singh, A., Bengrine, A., Nguyen-Khac, E., Naassila, M., and Bouhlal, H. (2018). Signaling lymphocytic activation molecules Slam and cancers: friends or foes? Oncotarget *9*, 16248-16262.

Goel, D., and Bhatnagar, R. (2012). Intradermal immunization with outer membrane protein 25 protects Balb/c mice from virulent B. abortus 544. Mol Immunol *51*, 159-168.
Goel, D., Rajendran, V., Ghosh, P.C., and Bhatnagar, R. (2013). Cell mediated immune response after challenge in Omp25 liposome immunized mice contributes to protection against virulent Brucella abortus 544. Vaccine *31*, 1231-1237.
Gorvel, J.P., and Moreno, E. (2002). Brucella intracellular life: from invasion to intracellular replication. Veterinary Microbiology *90*, 281-297.
Grillo, M.J., Blasco, J.M., Gorvel, J.P., Moriyon, I., and Moreno, E. (2012). What have we learned from brucellosis in the mouse model? Vet Res *43*, 29.
Gutierrez-Jimenez, C., Hysenaj, L., Alfaro-Alarcon, A., Mora-Cartin, R., Arce-Gorvel, V., Moreno, E., Gorvel, J.P., and Barquero-Calvo, E. (2018). Persistence of Brucella abortus in the Bone Marrow of Infected Mice. J Immunol Res *2018*, 5370414.
Jubier-Maurin, V., Boigegrain, R.A., Cloeckaert, A., Gross, A., Alvarez-Martinez, M.T., Terraza, A., Liautard, J., Kohler, S., Rouot, B., Dornand, J.*, et al.* (2001). Major outer membrane protein Omp25 of Brucella suis is involved in inhibition of tumor necrosis factor alpha production during infection of human macrophages. Infection and Immunity *69*, 4823-4830.
Ma, C., Wang, N., Detre, C., Wang, G., O'Keeffe, M., and Terhorst, C. (2012). Receptor signaling lymphocyte-activation molecule family 1 (Slamf1) regulates membrane fusion and NADPH oxidase 2 (NOX2) activity by recruiting a Beclin-1/Vps34/ultraviolet radiation resistance-associated gene (UVRAG) complex. J Biol Chem *287*, 18359-18365.
Macedo, G.C., Magnani, D.M., Carvalho, N.B., Bruna-Romero, O., Gazzinelli, R.T., and Oliveira, S.C. (2008). Central role of MyD88-dependent dendritic cell maturation and proinflammatory cytokine production to control Brucella abortus infection. J Immunol *180*, 1080-1087.
Manterola, L., Guzman-Verri, C., Chaves-Olarte, E., Barquero-Calvo, E., de Miguel, M.J., Moriyon, I., Grillo, M.J., Lopez-Goni, I., and Moreno, E. (2007). BvrR/BvrS-controlled outer membrane proteins Omp3a and Omp3b are not essential for Brucella abortus virulence. Infect Immun *75*, 4867-4874.
Martin-Martin, A.I., Caro-Hernandez, P., Sancho, P., Tejedor, C., Cloeckaert, A., Fernandez-Lago, L., and Vizcaino, N. (2009). Analysis of the occurrence and distribution of the Omp25/Omp31 family of surface proteins in the six classical Brucella species. Vet Microbiol *137*, 74-82.
Martirosyan, A., Moreno, E., and Gorvel, J.P. (2011). An evolutionary strategy for a stealthy intracellular Brucella pathogen. Immunological Reviews *240*, 211-234.
Martirosyan, A., Perez-Gutierrez, C., Banchereau, R., Dutartre, H., Lecine, P., Dullaers, M., Mello, M., Salcedo, S.P., Muller, A., Leserman, L.*, et al.* (2012). Brucella beta 1,2 cyclic glucan is an activator of human and mouse dendritic cells. PLoS Pathog *8*, e1002983.
Mattiuz, R., Wohn, C., Ghilas, S., Ambrosini, M., Alexandre, Y.O., Sanchez, C., Fries, A., Vu Manh, T.P., Malissen, B., Dalod, M.*, et al.* (2018). Novel Cre-Expressing Mouse Strains Permitting to Selectively Track and Edit Type 1 Conventional Dendritic Cells Facilitate Disentangling Their Complexity in vivo. Front Immunol *9*, 2805.

Moreno, E. (2014). Retrospective and prospective perspectives on zoonotic brucellosis. Front Microbiol *5*, 213.
Naparstek, E., Block, C.S., and Slavin, S. (1982). Transmission of brucellosis by bone marrow transplantation. Lancet *1*, 574-575.
Oguro, H., Ding, L., and Morrison, S.J. (2013). SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. Cell Stem Cell *13*, 102-116.
Papadopoulos, A., Gagnaire, A., Degos, C., de Chastellier, C., and Gorvel, J.P. (2016). Brucella discriminates between mouse dendritic cell subsets upon in vitro infection. Virulence *7*, 33-44.
Papadopoulos, A., and Gorvel, J.P. (2015). Subversion of mouse dendritic cell subset function by bacterial pathogens. Microbial Pathogenesis *89*, 140-149.
Pappas, G., Akritidis, N., Bosilkovski, M., and Tsianos, E. (2005). Brucellosis. N Engl J Med *352*, 2325-2336.
Pasquevich, K.A., Estein, S.M., Garcia Samartino, C., Zwerdling, A., Coria, L.M., Barrionuevo, P., Fossati, C.A., Giambartolomei, G.H., and Cassataro, J. (2009). Immunization with recombinant Brucella species outer membrane protein Omp16 or Omp19 in adjuvant induces specific CD4+ and CD8+ T cells as well as systemic and oral protection against Brucella abortus infection. Infect Immun *77*, 436-445.
Pasquinelli, V., Quiroga, M.F., Martinez, G.J., Zorrilla, L.C., Musella, R.M., Bracco, M.M., Belmonte, L., Malbran, A., Fainboim, L., Sieling, P.A.*, et al.* (2004). Expression of signaling lymphocytic activation molecule-associated protein interrupts IFN-gamma production in human tuberculosis. J Immunol *172*, 1177-1185.
Rethi, B., Gogolak, P., Szatmari, I., Veres, A., Erdos, E., Nagy, L., Rajnavolgyi, E., Terhorst, C., and Lanyi, A. (2006). SLAM/SLAM interactions inhibit CD40-induced production of inflammatory cytokines in monocyte-derived dendritic cells. Blood *107*, 2821-2829.
Robinson-Dunn, B. (2002). The microbiology laboratory's role in response to bioterrorism. Arch Pathol Lab Med *126*, 291-294.
Salcedo, S.P., Marchesini, M.I., Degos, C., Terwagne, M., Von Bargen, K., Lepidi, H., Herrmann, C.K., Lacerda, T.L.S., Imbert, P.R.C., Pierre, P.*, et al.* (2013). BtpB, a novel Brucella TIR-containing effector protein with immune modulatory functions. Frontiers in Cellular and Infection Microbiology *3*.
Salcedo, S.P., Marchesini, M.I., Lelouard, H., Fugier, E., Jolly, G., Balor, S., Muller, A., Lapaque, N., Demaria, O., Alexopoulou, L.*, et al.* (2008). Brucella control of dendritic cell maturation is dependent on the TIR-Containing protein btp1. Plos Pathogens *4*.
Sansonetti, P.J., and Di Santo, J.P. (2007). Debugging how bacteria manipulate the immune response. Immunity *26*, 149-161.
Schlitzer, A., Sivakamasundari, V., Chen, J., Sumatoh, H.R., Schreuder, J., Lum, J., Malleret, B., Zhang, S., Larbi, A., Zolezzi, F.*, et al.* (2015). Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. Nat Immunol *16*, 718-728.

Sieira, R., Comerci, D.J., Sanchez, D.O., and Ugalde, R.A. (2000). A homologue of an operon required for DNA transfer in Agrobacterium is required in Brucella abortus for virulence and intracellular multiplication. J Bacteriol *182*, 4849-4855.
Skendros, P., Pappas, G., and Boura, P. (2011). Cell-mediated immunity in human brucellosis. Microbes Infect *13*, 134-142.
Sowa, B.A., Kelly, K.A., Ficht, T.A., Frey, M., and Adams, L.G. (1991). SDS-soluble and peptidoglycan-bound proteins in the outer membrane-peptidoglycan complex of Brucella abortus. Vet Microbiol *27*, 351-369.
Tuon, F.F., Gondolfo, R.B., and Cerchiari, N. (2017). Human-to-human transmission of Brucella - a systematic review. Trop Med Int Health *22*, 539-546.
van Driel, B.J., Liao, G., Engel, P., and Terhorst, C. (2016). Responses to Microbial Challenges by SLAMF Receptors. Front Immunol *7*, 4.
Viadas, C., Rodriguez, M.C., Sangari, F.J., Gorvel, J.P., Garcia-Lobo, J.M., and Lopez-Goni, I. (2010). Transcriptome analysis of the Brucella abortus BvrR/BvrS two-component regulatory system. PLoS One *5*, e10216.
von Bargen, K., Gorvel, J.P., and Salcedo, S.P. (2012). Internal affairs: investigating the Brucella intracellular lifestyle. FEMS Microbiol Rev *36*, 533-562.
Yurchenko, M., Skjesol, A., Ryan, L., Richard, G.M., Kandasamy, R.K., Wang, N., Terhorst, C., Husebye, H., and Espevik, T. (2018). SLAMF1 is required for TLR4-mediated TRAM-TRIF-dependent signaling in human macrophages. J Cell Biol *217*, 1411-1429.
Zhao, Y., Hanniffy, S., Arce-Gorvel, V., Conde-Alvarez, R., Oh, S., Moriyon, I., Memet, S., and Gorvel, J.P. (2018). Immunomodulatory properties of Brucella melitensis lipopolysaccharide determinants on mouse dendritic cells in vitro and in vivo. Virulence *9*, 465-479.