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

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Abstract
The strategies by which intracellular pathogenic bacteria manipulate innate immunity to establish chronicity are poorly understood. Here, we show that *Brucella 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 (DCs) 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.

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

1 | 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 & Medzhitov, 2009; Sansonetti & Di Santo, 2007). Targeting the signalling triggered by pattern recognition receptors in antigen-presenting cells is one of them (Byndloss & 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.
Brucella spp. are Gram-negative facultative intracellular coccobi, which are the causative agent of brucellosis, a worldwide re-emerging zoonosis affecting live stock (bovine for Brucella abortus, ovine and caprine for Brucella melitensis, and swine for Brucella suis) and wild life with serious economic loss, as Brucella-infected animals undergo spontaneous abortion and infertility (Corbel, 1997; Cutler, Whaitmore, & Commander, 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, Akritidis, Bosilkovski, & Tsianos, 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 nonphagocytic or phagocytic cells, and as such, evade host immune system defence mechanisms (Byndloss & Tsolis, 2016a; Gorvel & Moreno, 2002; Martirosyan, Moreno, & Gorvel, 2011; von Bargen, Gorvel, & Salcedo, 2012). These properties may account for human brucellosis particularities (Skendros, Pappas, & Boura, 2011).

Dendritic cells (DCs) that display a higher susceptibility than macrophages to Brucella infection (Billard, Cazevieille, Dornand, & Gross, 2005) are essential for the induction of innate and adaptive immune responses against this pathogen (Avila-Calderon et al., 2019; Macedo et al., 2008). A number of bacterial effectors, including BtpA and BtpB proteins (Salcedo et al., 2008, 2013), and a noncanonical lipopolysaccharide (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 (Δomp25) induce human monocyte (mo)-DC and macrophage activation, which results in tumor necrosis factor alpha (TNF-α) and IL-12 secretion (Billard, Dornand, & Gross, 2007; Jubier-Maurin et al., 2001). In vivo, the role of Brucella Omp25 is controversial because B. abortus or B. melitensis Δomp25 mutants are either attenuated (Edmonds, Cloeckaert, & Elzer, 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 & Tsolis, 2016a; Commander et al., 2010; Commander, Spencer, Wren, & MacMillan, 2007; Goel & Bhatnagar, 2012; Goel, Rajendran, Ghosh, & Bhatnagar, 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 p1.2 cyclic glucan (CjG; Degos, Gagnaire, Banchereau, Moriyon, & Gorvel, 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 haematopoietic cells only and constitutes a self-ligand that triggers T-cell activation (Cannons, Tangye, & Schwartzberg, 2011; Fouquet et al., 2018). SLAMF1 is involved in immune responses against various pathogens (van Driel, Liao, Engel, & Terhorst, 2016). Identified as the entry receptor for measles virus, it controls DC and T-cell responses along viral infection. In macrophages, SLAMF1 binds Salmonella typhimurium and Escherichia 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 Mycobacterium tuberculosis and Trypanosoma cruzi infections (Calderon et al., 2012; Pasquinelli et al., 2004). Its role in brucellosis has not yet been 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 unrecoignised 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.

## 2 | RESULTS

### 2.1 | Brucella replication and persistence in BMDCs are independent of Omp25 and SLAMF1

In a previous transcriptomic study, we were intrigued by the strong upregulation of SLAMF1 gene expression in human mo-DCs treated with B. abortus cyclic p1.2-glucan (CjG; Degos et al., 2015; Table S1). Because 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 bone marrow-derived DCs (BMDCs) in vitro. BMDCs were challenged for 24 hr with B. abortus wild type (Ba WT) or a Omp25-defective mutant (Ba Δomp25) or its complemented mutant strain that stably re-expressed Omp25 (Ba Δomp25pBBR4omp25) or mock-treated or treated with E. coli LPS or CjG, 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 CjG- or E. coli LPS-treated cells, was observed with all types of infected samples compared with 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 Δomp25 mutant within BMDCs and compared it with that of Ba WT, Δomp25pBBR4omp25, or ΔvirB strains. The Ba Δomp25 mutant replicated intracellularly in BMDCs at a rate comparable with that of WT or Δomp25pBBR4omp25 strains in contrast to a nonreplicative strain, the virB mutant (Figure 2a), which persisted for 48 hr only (Sieira, Comerci, Sanchez, & Ugalde, 2000). The Δomp25 strain replicated within the endoplasmic reticulum 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.

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

We then characterised by flow cytometry the phenotype of BMDCs at 24 hr p.i. In Ba WT-infected BMDCs, DC activation, reflected by CD80, CD86, and CD40 costimulatory molecule expression levels (Figure 1a), was low compared with that of E. coli LPS-treated control cells (Figure 3a), as reported (Conde-Alvarez et al., 2012). However, Ba Δomp25-infected BMDCs exhibited significantly higher expression levels, although still below those of E. coli LPS-treated cells. The rescue of the Δomp25 mutant phenotype by the complemented strain (Δomp25pBBR4omp25) 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 ΔOmp25 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 Δomp25-infected BMDCs displayed much higher levels of TNF-α, interferon gamma, 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 Δomp25 strain (Figure 3b). The higher cytokine secretion of the Δomp25 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.

2.3 | 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

**FIGURE 1** In Brucella abortus-infected dendritic cells (DCs), SLAMF1 overexpression is Omp25-independent. (a) Flow cytometry characterisation of DCs in vitro upon B. abortus infection. Representative FACS profiles of C57BL/6j bone marrow-derived DCs 24 hr after mock-treatment or infection with B. abortus wild type (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 bone marrow-derived DCs 24 hr after mock treatment, stimulation with Escherichia coli LPS or B. abortus C3G, or infection with Ba WT or Ba Δomp25 or Δomp25pBBR4omp25 mutant strains; 3–5 points per group, n = 3. Mean ± SD from pooled data. Significant differences from Ba WT-infected DCs. *p < .01; **p < .001. ns, nonsignificant. Brown-Forsythe ANOVA with Welch’s correction
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 because 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.

### 2.4 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 hr p.i. by confocal immunofluorescence (Figure 5). BMDCs infected with *B. abortus* wild type (Ba WT), Δomp25, Δomp25pBBR4omp25, or ΔvirB strains. Mean ± SD is shown, n = 4. (b) Representative confocal microscopy images of C57BL/6J BMDCs infected with Ba WT or Δomp25 strains, fixed at 24 hr p.i., and stained for calnexin (endoplasmic reticulum staining, red) and anti-*Brucella* LPS (green). Scale bar of 10 μm. 50 cells per group, n = 4. (c) Kinetics of bacterial loads for wt or Slamf1−/− BMDCs infected with Ba WT, Δomp25, or Δomp25pBBR4omp25 strains. Mean ± SD is shown, n = 4. Mean ± SD from pooled data. Significant differences from Ba WT-infected DCs. ***p < .001. Absence of p value, nonsignificant. Brown-Forsythe ANOVA with Welch’s correction.

**FIGURE 2** In infected dendritic cells (DCs), *Brucella abortus* replication is independent of both Omp25 and SLAMF1. (a) Kinetics of bacterial loads for bone marrow-derived DCs (BMDCs) infected with *Brucella abortus* wild type (Ba WT), Δomp25, Δomp25pBBR4omp25, or ΔvirB strains. Mean ± SD is shown, n = 4. (b) Representative confocal microscopy images of C57BL/6J BMDCs infected with Ba WT or Δomp25 strains, fixed at 24 hr p.i., and stained for calnexin (endoplasmic reticulum staining, red) and anti-*Brucella* LPS (green). Scale bar of 10 μm. 50 cells per group, n = 4. (c) Kinetics of bacterial loads for wt or Slamf1−/− BMDCs infected with Ba WT, Δomp25, or Δomp25pBBR4omp25 strains. Mean ± SD is shown, n = 4. Mean ± SD from pooled data. Significant differences from Ba WT-infected DCs. ***p < .001. Absence of p value, nonsignificant. Brown-Forsythe ANOVA with Welch’s correction.
FIGURE 3  Brucella abortus infection inhibits dendritic cell (DC) activation in an Omp25-SLAMF1-dependent mode. (a) DC costimulatory molecule levels of expression (mean of fluorescence intensity, MFI) from wt or Slamf1−/− bone marrow-derived DCs mock treated, stimulated with Escherichia coli LPS or infected with B. abortus wild type (Ba WT), Δomp25, or Δomp25pBBR4omp25 strains for 24 hr. 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−/− bone marrow-derived DCs mock treated or infected with Ba WT or Δomp25 for 24 hr. n = 3. Mean ± SD from pooled data. Significant differences from Ba WT-infected DCs. *p < .05; **p < .01; ***p < .001. Absence of p value or ns, nonsignificant. Brown-Forsythe ANOVA with Welch's correction.
**FIGURE 4**  
*Brucella 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 dendritic cells (DCs). Confocal micrographs of wt or *Slamf1*−/− bone marrow-derived DCs mock infected or infected with *B. abortus* wild-type, Δomp25, or Δomp25pBBR4omp25 for 2 hr, 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 *B. abortus* wild-type. *p < .05; ***p < .001. Absence of p value, nonsignificant. Brown-Forsythe ANOVA with Welch’s correction.
2.5 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, Blasco, Gorvel, Moriyon, & Moreno, 2012). Spleens were first assessed 8 days after intraperitoneal (i.p.) challenge (Figure 6a) during the acute phase of infection. A significant increase in spleen weight of Δomp25-infected wt mice or Slamf1−/− mice compared with 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 Δomp25-infected 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 immunocompromised 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 Δomp25 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 Δomp25 mutant). As expected, infected animals in a wt background showed no effect on mouse viability. The reduced survival of Δomp25-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.

2.6 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 Δomp25 mutant were significantly heavier than that of mice infected with Ba WT strain and similar to those of Ba WT-infected or Δomp25-infected Slamf1−/− mice raised the possibility of in vivo 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-day p.i. (Figure 7b). Absolute numbers of cDC1, cDC2, and plasmacytoid DC were slightly augmented upon infection with Ba WT or Δomp25pBBR4omp25 strains compared with 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 Δomp25 of Slamf1−/− mice. We next asked whether this in vivo modulation of DC splenic subpopulations by the Omp25-dependent engagement of SLAMF1 by

![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 i.p. injection with PBS (Mock), Brucella abortus wild type (Ba WT) or Δomp25 or Δomp25pBBR4omp25 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, nonsignificant. Brown-Forsythe ANOVA with Welch’s correction. (b) Survival curve of wt (−) or Ifng−/− (−) mice infected with Ba WT (plain lines) or Δomp25 (dashed lines) strains. Five 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, nonsignificant. Mantel Cox test](image-url)
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 Δomp25 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.

2.7 | 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 Δomp25 mutant or Slamf1−/− mice infected with either Ba strains compared with that in wt mice infected with Ba WT. The Δomp25 mutant phenotype was rescued by the complemented strain Δomp25pBBR4omp25, clearly demonstrating the requirement of Omp25 for optimal settling of Brucella at the chronic phase. The comparable phenotype of Δomp25 mutant in wt mice and of the Ba WT and mutant strains in Slamp1−/− mice further supported the assumption that Omp25 operates through the engagement of the SLAMF1 receptor.

3 | DISCUSSION

In this study, we identify a role for SLAMF1 in establishment of chronic Brucella infection and discover a previously unrecognised interaction partner, B. abortus Omp25, in addition to the known
Measles virus, *Salmonella 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* lecitin FimH (van Driel et al., 2016). Future work will determine if other features of *Brucella* are recognised 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 (Eisenschink, Houle, & Hoffmann, 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, Verger, Grayon, Zygmun, & Grepinet, 1996). Anti-Omp25 antibodies reversed the inhibition of TNF-α elicited by *Brucella 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 3b and S3), which also requires SLAMF1, as shown by using SLAMF1-deficient cells or SLAMF1-blocking peptide (Figures 3b and S2). Variations in the apparent molecular sizes of Omp proteins have been attributed to a possible association with peptidoglycan of different sizes (Cloeckaert, Zygmun, de Wergifosse, Dubray, & Limet, 1992; Sowa, Kelly, Ficht, Frey, & Adams, 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 also has the ability to make direct contact with the SLAMF1 receptor at the surface of DCs, 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, Niazi, Sieling, Cheng, & Modlin, 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 costimulatory 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 Δomp25 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 downregulated inflammation, without affecting bacterial burden during acute infection. This was exemplified by the earlier death of Ba Δomp25-infected mice compared with 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 & Tsolis, 2016b). When the Omp25/SLAMF1 axis is missing, immune cells might not switch their 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, 2013). BtpA and BtpB interact via their Toll IL-1 receptor domain with the Toll-like receptor signalling complex after being exported from bacterial membranes and translocated to the host cell cytosol (Salcedo et al., 2008, 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, Pasqual, & Victoria, 2019), as SLAMF1 is recognised as a costimulatory molecule between DC and T cells (Cocks et al., 1995). Because 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 haemophilic interactions. SLAMF1 harbours in its cytoplasmic tail immunoreceptor tyrosine-based switch motifs, used by phosphatases and kinases, recruited through the SLAM adaptor protein, to control T-cell activation in a phospho-tyrosine-dependent mode (Cannons et al., 2011; Detre, Keszei, Romero, Tsokos, & Terhorst, 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 negative bacterium within infected DCs and define the transduction pathways involved and their interplay.

4 | EXPERIMENTAL PROCEDURES

4.1 | Ethics

Animal experimentation was conducted in strict compliance with good animal practice as defined by the French animal welfare bodies (Law 87–848 dated October 19, 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 minimise suffering during animal handling and experimentation.

4.2 | Mice

Six- to ten-week-old female C57BL/6J mice from Charles River, *Slamf1<sup>1<sup>−/−</sup></sup>* mice (kindly provided by Yusuke Yanagi; Davidson et al., 2004) or *Ifng<sup>−/−</sup>* (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 1 × 10<sup>6</sup> CFU for each *Brucella* strain. For survival experiments, mice were weighed every 2 days; when reaching a weight loss of 30%, they were sacrificed.

4.3 | Bacterial strains

*B. abortus* smooth virulent strain 2,308 and the derived strain devoid of Omp25, Δomp25<sup>a</sup>, (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* Δomp25 strain, Δomp25<sup>c</sup>BBR4omp25<sup>c</sup>, are presented below. *Brucella* strains were grown on tryptone soya agar plates, supplemented with kanamycin 25 µg ml<sup>−1</sup> for the Δomp25 strain or kanamycin and ampicillin 50 µg ml<sup>−1</sup> for the Δomp25<sup>c</sup>BBR4omp25<sup>c</sup> strain. For infection, strains were grown for 16 hr approximately at 37°C under shaking in tryptic soy broth (TSB), in presence of the appropriate antibiotics when required, until the OD at 600 nm reached 1.8. All *Brucelloae* were kept, grown, and used under strict biosafety containment conditions all along experiments in the BSL3 facility, Marseille. For subcloning of SLAMF1 and CD90 constructs, TOP10 thermo-competent E. coli bacteria were used. Liquid cultures were incubated for 16 hr in Luria broth (LB) at 37°C under constant shaking and in presence of the adequate antibiotics. Solid cultures were grown onto Luria broth agar.

4.4 | Construction of the complemented *B. abortus* Δomp25<sup>c</sup>BBR4omp25<sup>c</sup> strain

The *B. abortus* omp25<sup>a</sup> gene was amplified by polymerase chain reaction (PCR) using HiFi Taq polymerase according to manufacturer’s instructions with primers described in Table S2. Seven microlitres of the PCR product were used for A tailing in presence of 1X Taq Buffer, 0.2 mM of ATP, 5 units of Taq during 30 min at 70°C. Three microlitres 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. One microgram 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 hr at 16°C. Five microlitre 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 apr containing pBBR-MCS4-omp25 were added to 1 ml of a stationary culture of B. abortus Δomp25. After two washes in TSB, the pellet was resuspended in 100 μl of TSB and plated for 4 hr at 37°C in tryptone soya agar. Bacteria were then re-isolated in a plate containing ampicillin (50 μg ml⁻¹), kanamycin (25 μg ml⁻¹), and nalidixic acid (5 μg ml⁻¹) allowing the isolation of the B. abortus Δomp25pBBR4omp25 strain.

4.5 | Cell culture

BMDCs were prepared from 6- to 8-week-old C57BL/6J or Slamf1⁻/⁻ female femurs and tibias as previously described (Papadopoulos, Gagnaire, Degos, de Chastellier, & Gorvel, 2016). Briefly, bone ends were cut off, and bone marrow was flushed with RPMI 1640 supplemented with 5% FBS and 50 μg ml⁻¹ of gentamicin to kill extracellular bacteria. Thereafter, antibiotics concentration was decreased to 20 μg ml⁻¹ of gentamicin to kill extracellular bacteria. Thereafter, antibiotics concentration was decreased to 20 μg ml⁻¹ of gentamicin to kill extracellular bacteria. Thereafter, antibiotics concentration was decreased to 20 μg ml⁻¹ of gentamicin to kill extracellular bacteria. After two washes in TSB, the pellet was resuspended in 100 μl of TSB and plated for 4 hr at 37°C in tryptone soya agar. Bacteria were then re-isolated in a plate containing ampicillin (50 μg ml⁻¹), kanamycin (25 μg ml⁻¹), and nalidixic acid (5 μg ml⁻¹) allowing the isolation of the B. abortus Δomp25pBBR4omp25 strain.

4.6 | Cell culture infection

BMDCs were infected at a multiplicity of infection of 30:1. Bacteria were centrifuged onto cells at 400g for 10 min at 4°C and then incubated for 30 min at 37°C with 5% CO₂. Cells were washed twice with medium and incubated for 1 hr in medium containing 100 μg ml⁻¹ of gentamicin to kill extracellular bacteria. Thereafter, antibiotics concentration was decreased to 20 μg ml⁻¹ of gentamicin to kill extracellular bacteria. Thereafter, antibiotics concentration was decreased to 20 μg ml⁻¹ of gentamicin to kill extracellular bacteria. To monitor bacterial intracellular survival, infected cells were washed 3 times in 1x PBS and lysed with 0.1% Triton X-100 in H₂O. Serial dilutions were plated in triplicates onto TSB agar to enumerate CFUs after 3 days at 37°C.

4.7 | Construction of Myc-SLAMF1(exons 2–3) and Myc-CD90(exons 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.

4.8 | Expression and purification of Myc-SLAMF1 exons 2–3 or Myc-CD90 exons 2–3

Ten micrograms of Myc-SLAMF1 or Myc-CD90 expression vectors were transfected into COS-7 cells using Fugene 6 (Promega) according to manufacturers’ instructions. Forty-eight hours after transfection, cells were harvested and lysed into 1x PBS, 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.

4.9 | 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, and pH 7.4. To produce Omp25, the complete sequence of B. abortus omp25 gene (GenBank_X79284.1; de Wengifosse, Lintermans, Limet, & Cloeckaert, 1995) was synthetized and subcloned into pET22b+ (Novagen) in frame with 6x His-tag (Genscript). Expression and purification were performed as reported (Goel & Bhatnagar, 2012). Recombinant purified Omp25 was finally refolded and stored at –20°C in refolding buffer (50 mM TrisHCl, 1 M NaCl, 0.2 mM DTT, 0.1 mM EDTA, 0.5 M L-Ariginine-HCl, 10% Glyc- erol, 0.15 M Urea, and 3.6 mM Imidazole).

4.10 | Immunoprecipitation of Myc-SLAMF1 and Myc-CD90

Fifty microlitres of protein G-Dynabeads (Thermofisher) were coupled to 1 μg of anti-c-Myc antibody (9E10) during 1 hr at 4°C and then incubated with whole transfected COS-7 cell extracts in 1x PBS, 0.1% Triton X-100 and protease inhibitors for 1 hr at 4°C. After washes in 1x PBS, 0.1% NP-40, 0.1% FBS, and protease inhibitor cocktail, Myc-SLAMF1 and Myc-CD90 immunoprecipitated complexes were incubated 1 hr with 1 or 0.1 ng of purified recombinant B. abortus Omp25 or Omp19 at 4°C. Washes in 1x PBS, 0.5 M NaCl, 0.001% sodium dodecyl sulfate were then performed to eliminate nonspecific interactions. Samples were heated at 70°C in Laemmli buffer and centrifuged for 10 min at 14,000g. Supernatants were denatured at 95°C for 5 min, prior to loading on 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis. 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.
4.11 | 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.

4.12 | Quantitative PCR

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

4.13 | Cytokine dosage

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

4.14 | 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 1x PBS and once in 1x PBS. 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 median of fluorescence intensity measurements. For in vivo experiments, DC subset identification proceeded for FACS analysis. For histology studies, organs 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 was filtered in 1× PBS, 5 mM EDTA, and 2% FBS. After removal of red cells by the red blood cell lysis buffer (eBioscience), single splenic cell suspensions were proceeded for FACS analyses. For histology studies, organs were harvested and placed into 10% formalin for 24 hr at 22°C before inclusion in paraffin. Slides were then stained with haematoxylin and eosin.

4.15 | 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 1x PBS, followed by 1-hr blocking with 2% BSA in PBS. Primary antibodies were incubated for 1 hr followed by two washes in 1x PBS, 45-min incubation with secondary antibodies, two washes in 1x PBS, and one wash in water before mounting with Prolong Gold (ThermoFisher). For the other immunofluorescence labelling, 2% BSA in 1x PBS was used for 1 hr to block nonspecific interactions. Then, primary antibodies were incubated for 30 min in 1x PBS, 0.1% saponin, and 0.1% horse serum. Coverslips with cells were then washed twice in 1x PBS and 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 1024 x 1024 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 five independent experiments, for a total of at least 250 DCs analysed.

4.16 | Mouse infection

Six- to eight-week-old female C57BL/6J (wild-type), Slamf1−/− or Ifng−/− mice were infected in the BSL3 facility by i.p. injection. 1 x 10⁶ CFU were injected into 200 μl of sterile endotoxin-free PBS for each mouse. Organs were harvested at 8-, 20-, or 30-day p.i., weighted and then dissociated into sterile 0.1% Triton X-100 diluted in H2O. Serial dilutions in sterile 1x PBS were used to count CFU. Serial dilutions were plated in triplicates onto TSB agar to enumerate CFUs after 3 days at 37°C. For cytokmetry 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 was filtered in 1× PBS, 5 mM EDTA, and 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 hr at 22°C before inclusion in paraffin. Slides were then stained with haematoxylin and eosin.

4.17 | Statistical analysis

Statistical analyses were done using the GraphPad Prism software. Brown-Forsythe ANOVA test, followed by variance analysis with the Welch test, was performed, except for TNF-α dosage, for which the multiple comparison Kruskal Wallis ANOVA test, followed by variance analysis with the Dunn test, was 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 < .05 (*p < .05; **p < .01; ***p < .001). All experiments were performed at least 3 times in triplicate otherwise indicated.
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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 and 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.

CONFLICT OF INTERESTS
The authors declare no competing interests.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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