Brucella discriminates between mouse dendritic cell subsets upon in vitro infection

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Abbreviations: BCV, Brucella containing-vacuole; BMDCs, Bone marrow-derived dendritic cells; Btp, Brucella TIR-containing protein; CCL2, C-C chemokine ligand 2; CCR, C-C chemokine receptor; CFUs, Colony forming units; DC, dendritic cell; ER, Endoplasmic reticulum; Flt3l, Fms-like tyrosine kinase 3 ligand; FL-DC, Flt3l BMDCs; G-DC, GMCSF BMDCs; G/15-DC, GMCSF + IL15 BMDCs; G/FL-DC, GMCSF + Flt3l BMDCs; GMCSF, Granulocyte macrophage colony-stimulating factor; IFN, Interferon; IL, Interleukin; iNOS, inducible isoform of Nitric oxide synthase; LPS, Lipopolysaccharide; MCP-1, Monocyte chemotactic protein 1; MHC, Major histocompatibility complex; MOI, Multiplicity of infection; NOS2, Nitric oxide synthase 2; pDC, plasmacytoid DC; RT, Room temperature; TipDC, TNFα and iNOS-producing DCs; TLR, Toll-like receptor; TNFα, Tumor Necrosis Factor α; TSA, Tryptic Soy Agar; TSB, Tryptic Soy Broth.

Brucella is a Gram-negative bacterium responsible for brucellosis, a worldwide re-emerging zoonosis. Brucella has been shown to infect and replicate within Granulocyte macrophage colony-stimulating factor (GMCSF) in vitro grown bone marrow-derived dendritic cells (BMDC). In this cell model, Brucella can efficiently control BMDC maturation. However, it has been shown that Brucella infection in vivo induces spleen dendritic cells (DC) migration and maturation. As DCs form a complex network composed by several subpopulations, differences observed may be due to different interactions between Brucella and DC subsets. Here, we compare Brucella interaction with several in vitro BMDC models. The present study shows that Brucella is capable of replicating in all the BMDC models tested with a high infection rate at early time points in GMCSF-IL15 DCs and Flt3l DCs. GMCSF-IL15 DCs and Flt3l DCs are more activated than the other studied DC models and consequently intracellular bacteria are not efficiently targeted to the ER replicative niche. Interestingly, GMCSF-DC and GMCSF-Flt3l DC response to infection is comparable. However, the key difference between these 2 models concerns IL10 secretion by GMCSF DCs observed at 48 h post-infection. IL10 secretion can explain the weak secretion of IL12p70 and TNFα in the GMCSF-DC model and the low level of maturation observed when compared to GMCSF-IL15 DCs and Flt3l DCs. These models provide good tools to understand how Brucella induce DC maturation in vivo and may lead to new therapeutic design using DCs as cellular vaccines capable of enhancing immune response against pathogens.

Introduction

Brucella is a Gram-negative facultative intracellular bacterium responsible for brucellosis, a worldwide re-emerging zoonosis. Brucella is able to infect a large number of mammals including land and aquatic animals. In animals, brucellosis leads to abortion and sterility responsible for serious economic losses.

Brucella can be transmitted to humans by ingestion of contaminated food products or through exposure to infected aerosolized particles. Brucellosis in humans consists in a wide variety of symptoms, from recurrent fever and influenza-like symptoms in the acute phase, to arthritis in most common cases of chronic infection. Chronic brucellosis may affect different organs and induce more serious forms of the disease like hepatitis or endocarditis.

Upon infection, Brucella translocates through mucosal barrier and is internalized by professional phagocytes. It has been demonstrated that Brucella acts as a stealthy pathogen and modulates host immune responses. Brucella is able to survive and persist inside infected cells by modification of its intracellular trafficking. Bacteria avoid fusion with lysosomal compartments and reside in a Brucella-containing vacuole (BCV) harboring endoplasmic reticulum (ER) markers as a safe replicative niche. Brucella lipopolysaccharide (LPS) is implicated in the control of host immunity against Brucella infection. It is known as a poor Toll-like receptor 4 (TLR4) agonist reducing toxicity and immune responses. Most of these studies have been carried out on macrophages but in 2005 Billard et al. showed for the first time that Brucella can invade and proliferate in human DCs. DCs constitute the most potent professional antigen-presenting cell linking innate and adaptive immunity.

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Since then, different studies have showed that Brucella efficiently proliferates within human, murine and bovine DCs.\(^{17-23}\) Interestingly, Brucella infected human DCs do not engage into a maturation process and are poor inducers of naive T cells.\(^{18}\) Brucella also interferes with murine DC maturation in particular via BtpA and BtpB, 2 TIR-containing proteins that inhibit TLR signaling.\(^{20,24-27}\) Weak DC maturation can also be related to Brucella non endotoxic LPS,\(^{28,29}\) IL10 production\(^{30}\) and interference with type 1 Interferon (IFN) pathway.\(^{31}\) Brucella infection in \textit{vivo} induces the recruitment of inducible isof orm of Nitric oxide synthase (iNOS)-positive cells expressing CD11b, Ly6C and major histocompatibility complex (MHC) II,\(^{32}\) comparable to Tumor Necrosis Factor $\alpha$ (TNF$\alpha$) and iNOS-producing DCs (TipDC) identified following \textit{Listeria monocytogenes} infection.\(^{33}\) Interestingly, Copin et al. demonstrated by in situ microscopy analysis that Brucella infection \textit{in vivo} induces splenic DC maturation and migration to the white pulp T cell area.\(^{34}\) All these studies highlight the implication of DCs in \textit{Brucella} pathogenesis.

Most of the \textit{in vitro} studies performed to date have made use of GMCSF to differentiate bone marrow-derived monocytes into DCs (BMDCs).\(^{35}\) We know now that murine DCs form a complex network of cells composed of different subtypes with phenotypic and functional differences.\(^{14,36}\) Moreover, bacteria can interact differently with each DC subset and as a result the immune response can be subset-specific (Papadopoulos A and Gorvel JP, submitted). Interestingly, deep characterization of human DCs showed that they resemble mouse DCs populations and equivalents can be found for each subset that have been studied.\(^{36}\)

In order to decipher the mechanisms of \textit{Brucella}-DC interaction we compared \textit{Brucella} infection in different murine BMDCs models. To this aim, we used GMCSF BMDCs (G-DC). Then, we studied 3 other models: 1) Flt3l BMDCs (FL-DC) that gives rise to 3 DC subsets including plasmacytoid DC (pDC) and equivalents of splenic CD8$^+\$ and splenic CD11b$^+\$ \(^{37,38}\) 2) GMCSF + Flt3l BMDCs (G/FL-DC). We chose this model because it has been demonstrated that Flt3l is continuously produced \(^{39}\) and GMCSF is highly produced upon pathogenic challenge and can alter the developmental outcome of murine DCs.\(^{40,41}\) G/FL-DC may correspond to DCs found upon \textit{in vivo} infection. 3) GMCSF + IL15 BMDCs (G/15-DC). We selected this model because IL15 is produced by a wide range of tissues in response to inflammatory stimuli and some studies showed that IL15 can convert monocytes into DCs.\(^{42-46}\)

### Materials and Methods

#### Bacterial strain

In this study, \textit{B. abortus} smooth virulent strain 2308 was grown on Tryptic Soy Agar (TSA) plates (Sigma Aldrich) for 7 d. For infection, 2mL of Tryptic Soy Broth (TSB) (Sigma Aldrich) were inoculated with one colony. Bacteria were grown overnight at 37°C under shaking until the OD (600nm) reached 1.8. All experiments with \textit{Brucella} were carried out in a BSL3 facility.

#### Mice

6- to 10-week-old wild type C57BL/6 female mice were obtained from Charles River Laboratories. All experiments were done in accordance with French and European guidelines for animal care.

#### \textit{In vitro} generation of BMDCs

BMDCs were prepared from 6–10 week-old C57BL/6 female femurs and tibias as previously described.\(^{20}\) Briefly, bone ends were cut off and marrow was flushed with RPMI medium (GIBCO) containing 5% FCS and 50 $\mu$M of 2-mercaptoethanol (Sigma). 3 x 10$^6$ cells were seeded onto 6-well plates in a medium containing either supernatant of the J558L GMCSF producing cell line (G-DC), both GMCSF and IL15 at 20 ng/mL (Miltenyi) (G/IL15-DC) or both GMCSF and supernatant of the Flt3l-producing B16 melanoma cell line (G/FL-DC). 1.5 x 10$^6$ cells were seeded in 6-well plates in a medium containing the supernatant of the Flt3l-producing B16 melanoma cell line only (FL-DC).

G-DC medium was changed at day 2.5. G/IL15-DC medium was changed at days 2 and 4. G/FL-DC and FL-DC medium was changed at day 4.

#### Infection of BMDCs

BMDCs infections were performed at a multiplicity of infection (MOI) of 30. Bacteria were centrifuged onto cells at 400 g for 10 min at 4°C and then incubated for 30 min at 37°C in a 5% CO2 incubator. Cells were washed twice with medium and then incubated for 1 h in medium containing 50 $\mu$g/ml gentamicin (Sigma Aldrich) at 37°C to kill extracellular bacteria. Thereafter, the antibiotic concentration was decreased to 10 $\mu$g/ml.

#### Intracellular replication assays

To monitor bacterial intracellular survival, infected cells were washed 3 times with PBS and lysed with 0.1% Triton X-100 in H$_2$O for 10 min and serial dilutions were plated in triplicate onto TSB agar and Colony forming units (CFUs) were scored after 3–4 d of culturing at 37°C.

#### Electron microscopy

Cells were fixed for 1h at room temperature with 2.5% glutaraldehyde in 0.1M Na-cacodylate buffer, pH 7.2 containing 0.1M sucrose, 5 mM CaCl$_2$ and 5 mM MgCl$_2$ and washed with complete cacodylate buffer, and post-fixed for 1 h at room temperature with 1% osmium tetroxide in the same buffer devoid of sucrose. They were washed with buffer, scraped off the dishes, concentrated in 2% agar in cacodylate buffer and treated for 1 h at room temperature with 1% uranyl acetate in Veronal buffer. Samples were dehydrated in a graded series of ethanol and embedded in Spurr resin. Thin sections (70 nm thick) were stained with 1% uranyl acetate in distilled water and then with lead citrate.

#### Flow cytometry

Cells were harvested and stained for 20 min at 4°C with antibodies mix. After a wash in PBS with 2% of FCS, cells were
stained with Fixable Viability Dye eFluor 506 (eBiosciences) for 10 min at room temperature to evaluate cell viability. Cells were then fixed for 20 min in 3.2 % PFA at RT. Antibodies used in flow cytometry are the following, CD11c-PeCy5.5 (1/1500), B220 BV605 (1/200) and MHC II (I-A/I-E) Al700 (1/800) from eBioscience, CD11b APC-Cy7 (1/800), CD86 BV650 (1/200), CD80-BV421 (1/800) and CD40-PeCy5 (1/400) from BioLegend, SIRPα Pe (1/200), CD24 Pe-Texas red (1/2000) and Annexin V APC (1/500) (BD).

G-DC, G/IL15-DC and G/FL-DC were gated based on the expression of CD11c and CD11b. Flt3l were separated in 3 sub-types: pDC expressing CD11c (intermediate) and B220 and negative for CD11b; CD11b+ DC equivalent expressing CD11c, SIRPα and CD11b and negative for CD24 and B220; CD8α+ equivalent expressing CD11c, CD24 and negative for CD11b, SIRPα and B220.

Flow cytometry was performed using a FACSLSRII (Becton Dickinson) and data were analyzed with BD FACSDIVA software.

RNA extraction and RT
Total RNAs were extracted from infected BMDCs using RNeasy Mini Kit (Qiagen) and following manufacturer’s instructions. cDNAs were generated by using Quantitech Reverse Transcription Kit (Qiagen) following manufacturer’s instructions.

qPCR
qPCR was performed with SYBR Green (Takara) following the manufacturer’s instructions in 7500 Fast Real-time PCR (Applied Biosystem). HPRT was used as a housekeeping gene to determine ∆Ct. Fold increase was compared between the control and the infected cells. Primers used in this study to amplify mouse cDNA are presented in the Table 1.

Cytokine measurement
Culture supernatants were analyzed by cytometric beads assay (CBA from BD, Mouse Inflammation kit) and ELISA from eBiosciences for IL1β, IL-12p40 and IL-12p70.

Table 1. qPCR primers

|     | FW                        | RV                        |
|-----|---------------------------|----------------------------|
| IL-12b | AAATTACTCGGAGGGTTCA       | ACAGAGACCGCTACCTCAAAT     |
| TNFa | CATCTTCTAAAATTCGAGGCAAA   | TGGGAGTAGACAAAGTGACACC    |
| IFNγ | TCAAGTGCAGTACAGTGAGAAGAA | TGGCTCTGCGAGGATTTCATG     |
| IL-6 | GAGGATACCTCCCAACAGACC     | AAATGGATCACACGCCAGGTTA    |
| IL1β | TCCAGGATAGGAGCATGACAC     | GAAGTCACAACACCGAGGTGA     |
| IL-10 | GGTGTCGACAGCTTATCGGA     | ACCTGCTGCAGCTGCTG        |
| CCL2 | GCTGCTGTCAACAGTTGC       | ATGGGATACATCTGCTGG        |
| IFNα | GAGACTTGGATCCGAGGAGAAGAGG | GCTGATCAGAACGCTCTGAGGTC   |
| IFNβ | GAAAGCGAAGGAGAAGGATT      | AAGCTTCTGGAATGAGAAGA      |
| CCR2 | GAAGAGGCGATTGACATTCC      | GTATGCGTGGATGAACTGGA      |
| CCR7 | GTGATGGCTCTCCTTGTCAT      | GAAGCACACCAGCACTGTACA     |
| NOS2 | CAGCTGGGCTGTAACACCTT      | CATTGGAAAGTGAGCGTTTCG     |

Statistics
All experiments were performed at least 5 independent times. Flow cytometry statistical analysis was performed using a ratio paired T test (paired and parametric) to compare mean of fluorescence of more than 100 000 cells in non-infected vs infected conditions. Cytokine secretion statistical analysis was performed with an unpaired T test to compare CFU counts. GMCSF BMDCs are used as a reference to compare other BMDCs model. P values > 0.05 were not consider significant, p values < 0.05 are represent with *, p values < 0.01 are represent with **, p values < 0.001 are represent with *** and p values < 0.0001 are represent with ****.

Figure 1. Brucella CFU counts and replication in different BMDCs models
BMDCs from C57BL/6 mice were infected at a MOI of 30. Extracellular bacteria were killed by antibiotic treatment and cells were recovered, lysed and plated to obtain intracellular bacterial counts per well at different time point. Each point represent at least 3 experiments. Statistical analysis were performed using an unpaired T test to compare CFU counts. GMCSF BMDCs are used as a reference to compare other BMDCs model. P values > 0.05 were not consider significant, p values < 0.05 are represent with *, p values < 0.01 are represent with **, p values < 0.001 are represent with *** and p values < 0.0001 are represent with ****.

(A) Results are expressed by number of CFU obtain at different time point. (B) Results are standardized to compare Brucella replication using CFU count 2 hours post-infection as a reference.
using a Wilcoxon test (paired and non-parametric) to compare non-infected vs infected conditions and a Mann-Whitney test (unpaired and non-parametric) to compare results between infected BMDCs models. P values over 0.05 were not considered as significant.

Results

Replication of Brucella in different BMDC models

We first tested the infection and proliferation efficiency of B. abortus in 4 different BMDC models. Cells were infected at an MOI of 30 and the intracellular bacteria were recovered at 2, 8, 24 and 48 h post-infection (Fig. 1A). Results showed that B. abortus can infect all the BMDC sub-types. CFU counts decreased for the first 8 h post-infection. This was followed by a proliferation phase until a plateau value was reached. Interestingly, at 2 h post-infection differences between DC sub-types was observed. Both G/15-DCs and FL-DCs were respectively 3 and 10 times more infected than G-DCs and G/FL-DCs. At late time points, these differences were lost and Brucella CFU scores were similar between the different DC models. We also determined the fold increase in the amount of bacteria with respect to the amount found at 2h post-infection (Fig. 2B). Results showed that Brucella replication is significantly more important in G-DCs and G/FL-DCs than in G/15-DCs and FL-DCs.

Fate of B. abortus in the different BMDC models

It has been shown in macrophages and G-DC that B. abortus escapes lysosomal compartments and fuses with ER membranes to establish a safe replicative niche.4-10

To characterize the compartment in which B. abortus was contained in the different BMDC models, infected cells were fixed and processed for electron microscopy at selected time point post-infection. The morphological appearance was analyzed on electron microscopy thin sections and BCV profiles were then examined for the presence or absence of ribosomes (arrows Fig. 2). BCVs harboring ribosomes indicated that they were ER-derived (left panel Fig. 2A). Some BCVs containing either one or several bacteria were free of ribosomes (right panel Fig. 2A). They seem to contain lysosomal material. The relative abundance of ER-derived BCV was evaluated on EM cell profiles at 24 and 48h post-infection (Fig. 2B). As previously described in G-BMDCs20 more than 90% of B. abortus were located in vacuoles surrounded by ribosomes. We obtained different results for all the other DC sub-types tested. Upon G/FL-DC infection, bacteria were mainly present in an ER-derived vacuoles but 20% of BCVs either

Figure 2. Intracellular localization in BMDCs analyzed by electronic microscopy
BMDCs from C57BL/6 mice were infected at a MOI of 30. 24h and 48h post-infection, cells were fixed and processed for Electron Microscopy analysis. The BCV morphological appearance was analyzed on electron microscopy thin sections either BCVs harboring ribosomes (A/arrows left panel). Some BCVs containing either one or several bacteria were free of ribosomes (A/right panel). The relative abundance of ER-derived BCV was evaluated on EM cell profiles at 24 and 48h post-infection (B). Combining three different experiment around 100 BCV were quantified for each time-point and results are expressed by a percentage.
contained lysosomal material or had not yet fused with ER membranes. Upon G/IL15-DC infection only 38% of BCV harbored ribosomes at 24 h post-infection and this percentage increased up to 70% at 48 h post-infection. For FL-DC, bacteria were frequently found in phagolysosomes-like vacuoles and only 20% of the BCV were surrounded by ribosomes. This percentage reached 45% at 48 h post infection. These results show that although the intracellular location of B. abortus varied according to the BMDC model, the intracellular survival was not affected.

**Acquisition of surface maturation markers**

To assess the phenotypic maturation of the different BMDCs models, we measured the expression of DC surface maturation markers by flow cytometry. We compared the mean of

![Figure 3](https://www.tandfonline.com)

**Figure 3.** Surface marker expression BMDCs from C57BL/6 mice were infected at a MOI of 30. 4h, 24h and 48h post-infection, cells were fixed and stained with antibodies for MHCII, CD86, CD80 and CD40. Results are presented in box plot and each point represent at least 5 experiments. Statistical analysis were performed using a ratio paired T test (paired and parametric) to compare mean of fluorescence of more than 100 000 cells in non-infected vs infected conditions. Statistical significance represent difference between infected and non-infected conditions. P values > 0.05 were not consider significant, p values < 0.05 are represent with *, p values < 0.01 are represent with **, p values < 0.001 are represent with *** and p values < 0.0001 are represent with ****.
fluorescence intensity ratio between non-infected and infected cells (Fig. 3).

Upon *B. abortus* infection, G-DC maturation markers were not significantly up-regulated at 24 h post-infection as it had previously been shown. At 48 h post-infection, costimulatory molecules were significantly upregulated but not MHCII molecules. G/FL-DC response to *B. abortus* infection was comparable to G-DC infection with a late maturation. Interestingly, G/IL15-DC infection induced a significant maturation from 24 h post-infection onwards. The FL-DC sub-type includes 3 different subsets: pDC, CD8α+ and CD11b+ equivalents. By using a combination of different markers we were able to separate them by flow cytometry and analyze the maturation level of each population. Flt3l pDC maturation marker upregulation was observed at 48 h post-infection and from 24 h onwards for CD40. Flt3l CD11b+ and CD8α+ BMDCs were activated for all of the co-stimulatory molecules from 24 h onwards. There was no effect on MHCII expression in CD11b+ BMDCs and a weak up-regulation in CD8α+ BMDCs.

Altogether, flow cytometry results showed that G-DC, G/FL-DC and FL-pDC were activated upon *B. abortus* infection only at 48 h post-infection. However, G/IL15-DC, Flt3l CD11b+ and CD8α+ BMDCs were activated from 24 h onwards. We show here that *B. abortus* can induce in vitro DC activation with different kinetics depending on the BMDC model. These results can be related with *Brucella* intracellular localization. The less bacteria are in the ER compartment, the more BMDCs are activated.

**BMDCs death**

To compare the effect of *B. abortus* infection on different BMDCs subsets we also decided to check the effect on cell death. To this aim, we used a combination of a dead cell staining and Annexin V labeling (Fig. 4). Annexin V stained phosphatidylserine in the outer leaflet, sign of the early stage of programmed cell death. Results show that none of the BMDC models were stained for the dead cell marker. However, all DCs except Flt3l pDC BMDCs and G/IL15-DCs were stained by Annexin V at 24 h post-infection. In addition, CD11b+ and CD8α+ Flt3l BMDCs and G/IL15-DCs were stained by Annexin V at 24 h post-infection. These results indicate that an early activation of DC induce an early Annexin V staining.

We then checked for BMDCs proliferation by measuring BrdU incorporation and no significant differences were obtained upon
B. abortus infection (data not shown).

Gene expression profile

To highlight some specific patterns, we analyzed BMDC gene expression profiles by comparing the fold increase in infected cells as compared to non-infected cells. Statistical analysis was performed by using the Comparative CT Method (ΔΔCT Method) given by: $2^{-\Delta\Delta CT}$. The dotted line represent a fold increase of 2, the statistical significant threshold in this method (Fig. 5).

At 2 h post-infection, IFNα, IL1β, nitric oxide synthase 2 (NOS2) and IL10 started to be upregulated in all BMDCs subsets. At 8, 24 and 48 h post-infection all genes tested were upregulated. IL12 and IL1β gene expression followed the same kinetics in all of the BMDCs, with an increase from 8 h to 24 h and a decrease at 48 h post-infection. However, we identified some important differences between DC subsets. G-DC infection induced a strong and specific IL10 upregulation at 48 h post-infection. This strong IL10 up-regulation may explain that G-DC are less activated than all the other BMDCs. Analysis of the expression of maturation markers and CFUs counts showed that G-DC and G/FL-DC respond in the same way to B. abortus infection. Gene expression of these 2 subsets showed similar kinetics except for IL10. It is noteworthy that gene upregulation was lower in G-DCs than in the other BMDC models for almost all of the gene tested. We previously showed that G/IL15-DC infection induced an early upregulation of surface maturation markers. Gene expression profile showed many differences with G-DC. Kinetics of expression was different for IFNγ, IFNβ, TNFα and IL6. IFNγ gene was overexpressed earlier as compared to all the model tested and was strongly down regulated at 48 h post-infection. IFNβ, TNFα and IL6 were expressed later than in other subsets with a peak at 24 h of infection for IFNβ and after 48 h of infection for TNFα and IL6. FL-DC gene overexpression was the highest observed for TNFα, IFNγ, IL6, IFNα, IFNβ, IL1β and NOS2. Again, these results are in line with all the previous results showing an early important FL-DC maturation.

We checked the expression of the chemokine C-C chemokine ligand 2 (CCL2) and its receptor C-C chemokine receptor (CCR) 2 (Fig. 6) as they correlate with DC maturation. A fold-increase of 2 represented by the dotted line shows the statistical significant threshold.

CCL2 also called monocyte chemotactic protein 1 (MCP-1) is essential for monocyte recruitment from bone marrow to inflammatory sites. Upon B. abortus infection in all the BMDCs, we found an increased expression of CCL2. However, we noticed different levels of up-regulation between these subsets. G-DC exhibited the lowest increase. In contrast infection of FL-DC was responsible for a 300-fold increase of the CCL2 expression as compared to the case of non-infected control cells. CCR2 expression is known to be down-regulated upon DC activation. Here, results showed that CCR2 expression significantly decreased at
48 h post-infection. In G/15-DC, CCR2 expression started to decrease at 24 h post-infection. Interestingly, G-DC presents the lowest down-regulation of CCR2.

Then we checked the expression of CCR7 (Fig. 6). This chemokine receptor has been identified as a key regulator of immune cells trafficking to lymph node, expressed upon DC maturation. Results showed a slight upregulation at 24 h post-infection in G-DC, G/FL-DC and G/15-DC. Interestingly, B. abortus infection induced a strong upregulation of CCR7 starting from 8 h post-infection in FL-DC.

Cytokine secretion
To assess the functional maturation of BMDCs, we also measured cytokine secretion by recovering BMDCs culture supernatants at 24 h and 48 h post-infection. We tested IL6, IL10, IL1β, IL12p40 and 70, IFNγ, TNFα and CCL2 (Fig. 7).
At 24 h and 48 h post-infection, TNFα, IL6, IL12p40, IL1β and CCL2 were significantly secreted by all BMDCs models.

At 24 h post-infection, only G/IL15-DC and FL-DC secreted IL12p70 and IFNγ. IL10 was not significantly secreted by any BMDCs. We can notice that G/IL15-DC secreted higher amounts of IL12p70, TNFα, IFNγ, IL1β and IL6 than all the other models.

At 48 h post-infection, G-DC secreted low levels of IL12p70 and G/FL-DC secreted IL12p70 and IFNγ. IL10 was significantly up-regulated IL10 production, as it has been shown analyzing gene expression. Interestingly, G/IL15-DC TNFα, IFNγ and IL6 secretion decreased when compared to 24 h post-infection time point.

**Discussion**

The present study shows that *Brucella* is capable of replicating in all the BMDCs models tested with a high infection rate at early...
In vivo, and proliferates. In this context, pro-inflammatory cytokine GMCSF to FL-DC inhibits pDC and CD8 compared G-DC and G/FL-DC function and concluded that adding This result is in agreement with previous studies, which com-
parable, with an exception concerning migration capacity.

We show that G-DC and G/FL-DC response to infection is comparable, with an exception concerning migration capacity. This result is in agreement with previous studies, which com-
pared G-DC and G/FL-DC function and concluded that adding GMCSF to FL-DC inhibits pDC and CD8$^+$ differentiation and induces production of CD11b$^+$ DC almost identical to G-
DC. $^{41,52}$ The key difference between these 2 models concerns IL10 secretion at 48 h post-infection. Interestingly, a study showed that IL10 inhibition in G-DC induces Brucella clearance with an increase in IL12p70 and TNF$\alpha$ secretion. $^{30}$ IL10 secretion can explain the weak secretion of these 2 cytokines in the G-
DC model and the low level of maturation observed when com-
pared to the other models G/15-DCs and FL-DCs.

Here, we show that G/15-DC maturation was followed by a strong pro-inflammatory cytokine gene up-regulation and a strong IFN$\gamma$ and IL12p70 production. These results can be cor-
related with literature in which G/15-DCs have been shown as great producers of IL12p70 and IFN$\gamma$. $^{53}$ Moreover, it is known that IFN$\gamma$ plays an important role in mediating Brucella resis-
tance, $^{54,56}$ but in G/15-DC model Brucella significantly survives and proliferates. In this context, pro-inflammatory cytokine secretion was not sufficient to impede bacterial replication. In vivo, we can expect an efficient stimulation of macrophages with a important IFN$\gamma$ production. To investigate this point, it will be interesting to test whether G/15-DC cytokine production can stimulate macrophages and induce a killing effect on Brucella.

Gene expression and cytokine secretion showed apparent discrepancies. This can be explain by at least 2 different aspects. It is known that mRNA and protein half-life can vary from minutes to days and, as a result, experimental quantification may not reflect the effective production. However, discrepancies observed can also be due to a real mechanism induced by Brucella infection. As already discussed in Martinoy et al. $^{57}$ a strong cyto-
kine synthesis may trigger the activation of the proteasome machinery leading to intracellular cytokine degradation and consequently the decrease of their secretion.

It has been shown that Brucella induces splenic DC migration and maturation. $^{34}$ We show that FL-DC infection increases the maturation of CD8$^+$ and CD11b$^+$ DCs and a strong CCR7 upregulation. As FL-DCs can be considered as splenic DC equival-
ents, we demonstrate here that FL-DC represents a good model to study Brucella interaction with splenic DC.

Altogether, this study may help to open a field of a wide range of studies on interactions between Brucella and DC subtypes.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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