Brucella suis Prevents Human Dendritic Cell Maturation and Antigen Presentation through Regulation of Tumor Necrosis Factor Alpha Secretion

Elisabeth Billard, Jacques Dornand, and Antoine Gross*

INSERM U431, CPBS UMR CNRS 5236 UMI UM2, F-34095 Montpellier, France

Received 7 May 2007/Returned for modification 21 June 2007/Accepted 2 July 2007

Brucella is a facultative intracellular pathogen and the etiological agent of brucellosis. In some cases, human brucellosis results in a persistent infection that may reactivate years after the initial exposure. The mechanisms by which the parasite evades clearance by the immune response to chronically infect its host are unknown. We recently demonstrated that dendritic cells (DCs), which are critical components of adaptive immunity, are highly susceptible to Brucella infection and are a preferential niche for the development of the bacteria. Here, we report that in contrast to several intracellular bacteria, Brucella prevented the infected DCs from engaging in their maturation process and impaired their capacities to present antigen to naïve T cells and to secrete interleukin-12. Moreover, Brucella-infected DCs failed to release tumor necrosis factor alpha (TNF-α), a defect involving the bacterial protein Omp25. Exogenous TNF-α addition to Brucella-infected DCs restored cell maturation and allowed them to present antigens. Two avirulent mutants of B. suis, B. suis bvrR and B. suis omp25 mutants, which do not express the Omp25 protein, triggered TNF-α production upon DC invasion. Cells infected with these mutants subsequently matured and acquired the ability to present antigens, two properties which were dramatically impaired by addition of anti-TNF-α antibodies. In light of these data, we propose a model in which virulent Brucella alters the maturation and functions of DCs through Omp25-dependent control of TNF-α production. This model defines a specific evasion strategy of the bacteria by which they can escape the immune response to chronically infect their host.

Brucella is a facultative intracellular α2-proteobacterium that induces chronic infections in a wide variety of mammals, including field ruminants, humans, and marine mammals. In addition to the attention received from its classification as a potential weapon for bioterrorism (41), this bacterium is principally known because of its ability to induce infectious abortion in domestic animals and because brucellosis is the most frequent anthropozoonosis (20). The three most infectious species in humans are B. melitensis, B. abortus, and B. suis. Infection occurs through inhalation of aerosols or ingestion of infected food. Following invasion of the lymphoid system, the bacteria develop within mononuclear phagocytes, and infected cells play a crucial role in the dissemination of the bacteria in specific locations of the body. Also known as Malta fever, human brucellosis consists of an acute infection, characterized by undulant fever and arthritis, which evolves in 30% of infected patients into a chronic phase with erratic recurrent fevers and localized infections, such as endocarditis, encephalitis, and spondylitis. Chronic brucellosis patients display a T lymphocytes (2, 3, 46). Therefore, the ability of Brucella to chronically infect human hosts seems to be related to its capacity to avoid establishment of a protective Th1-specific response (7, 19, 43, 55).

For several years, our laboratory and others have studied the interaction of Brucella with macrophages and identified several virulence mechanisms implicated in the interaction of Brucella with the innate immune system (1, 9, 10, 18, 21, 25, 29, 30, 39). Nevertheless, due to reduced implication of macrophages in the initiation of a specific immune response, the macrophage infection model is not suitable when the adaptive immune response is considered in the context of the Brucella-host interaction. Myeloid dendritic cells (DCs) have naturally emerged as interesting models. DCs serve as sentinels for the immune system; they ingest pathogens at the site of infection and migrate to secondary lymphoid organs, where they present pathogen-derived antigens to naïve T lymphocytes, thus initiating the specific immune response. Pathogens could target DCs during early stages of infection as a way of disabling and evading host immune responses. In a previous publication, we showed for the first time that human DCs are highly permissive cellular hosts for Brucella (5). In this report, we analyze the consequences of Brucella infection for DC physiology, particularly for maturation processes and antigen presentation to naïve T cells. Relationships with some bacterial proteins were studied, and the importance of DC infection for Brucella’s virulence strategy and the pathogenesis of human brucellosis is discussed.

MATERIALS AND METHODS

Bacteria. The Brucella strains used in this study are mutants of B. suis 1330. The strain referred to as the wild type (WT) constitutively expresses a green fluorescent protein (GFP). Every bacterial strain mentioned here has been de-
scribed in detail elsewhere (27, 30). Two B. suis mutants were used, in which the bvrR and omp25 genes were inactivated: B. suis bvrR (B. suis bscp31::GFP bvrR::Tn5Km2 [30]) and B. suis omp25 (B. suis Domp25::kan [27]). The smooth character of the Brucella strains used in this study was originally controlled by crystal violet staining and immunoblotting techniques, as previously mentioned (26). No differences were observed between B. suis 1330 GFP and B. suis 1330 bscp31::GFP (parental strain of the bvrR mutant) (30), so we present data for only one control in the figures for clarity. The GFP-expressing strain Escherichia coli S17.1 D3 (49) was a generous gift from A. Givaudan, INRA UMR 1133, Montpellier, France.

Antibodies and reagents. The anti-human tumor necrosis factor alpha (TNF-α) antibody (R&D Systems, Minneapolis, MN) was used at a concentration of 2.5 µg/ml, and recombinant human TNF-α (rhTNF-α) (Immunotools, Friesoythe, Germany) was used at a concentration of 10 ng/ml.

Most antibodies used for DC phenotypic analysis were purchased from BD Pharmingen, San Diego, CA; the exceptions were mouse anti-CCR7 (R&D Systems) and anti-HLA-ABC (Beckmann-Coulter).

DC preparation. Immature DCs were prepared from peripheral blood circulating monocytes obtained by centrifugation on Ficoll-Hypaque (Sigma, Lyon, France) of buffy coat from healthy donors provided by the Etablissement Français du Sang. CD14<sup>+</sup> monocytes were purified by magnetic bead-positive separation (Miltenyi Biotec, Paris, France) and then differentiated for 5 days in complete medium (RPMI 1640, 10% fetal calf serum, 50 µM β-mercaptoethanol, 500 U/ml interleukin-4 [IL-4], 1,000 U/ml granulocyte-macrophage colony-stimulating factor [both cytokines were obtained from Immunotools]) (5).

Infection experiments. Immature DCs were harvested, resuspended in RPMI medium plus 10% fetal calf serum, and infected for 1 h at 37°C with bacteria at concentrations corresponding to a CFU/DC ratio of 5:1. The cells were then washed in phosphate-buffered saline (Invitrogen) and reincubated in fresh medium supplemented with 50 µg/ml gentamicin in order to kill remaining extracellular bacteria (21). In these experimental conditions, the rates of infection of DCs with Brucella and E. coli were similar, 29.6% ± 2.6% and 31.12% ± 3.2%, respectively. This was described in a previous study in which fluorescence microscopy experiments were performed by using GFP-labeled bacteria and by

FIG. 1. Fluorescence-activated cell sorting analysis of DC maturation in response to infection by B. suis. Immature human DCs were infected with WT B. suis or E. coli for 48 h and then stained for maturation marker expression. Cytometry analysis histograms are presented for each surface molecule studied and show the results of 1 experiment that was representative of 22 independent experiments.
counting viable intracellular bacteria (5). For assessment of intracellular prolifera-
tion, infected cells (2 \times 10^5 cells/well) were washed and lysed at several times
postinfection (p.i.) in 0.1% Triton X-100 (Sigma). The number of intracellular
viable bacteria (in CFU per well) was determined by plating 10-fold serial
dilutions on tryptic soy agar plates.

**Maturation analysis.** At 48 h.p.i., DCs were labeled with mouse anti-human
monoclonal antibodies followed by a phycoerythrin-conjugated goat anti-mouse
polyclonal antibody (BD Pharmingen) and were analyzed with a FACS Calibur
cytometer (Becton Dickinson, San Jose, CA).

**Cytokine measurement.** For cytokine measurement, supernatants were col-
clected and concentrations of TNF-α and IL-12p70 were measured with an
OptEIA human enzyme-linked immunosorbent assay set (BD Pharmingen) or by
flow cytometry using a CBA Flex set (BD Biosciences).

**Antigen presentation to naive human T lymphocytes.** Human naïve CD4+ T
cells were prepared using an EasySep human naïve CD4+ T-cell enrichment kit
(Stem Cell Technologies) according to the manufacturer’s instructions. Naïve T
cells (CD3+ CD4+ CD45RA+) were stained intracellularly at 37°C in RPMI
medium containing 1 µM carboxyfluorescein diacetate succinimidyl ester
(CFSE) (Sigma-Aldrich), extensively washed in medium, and plated in a 96-well
plate (10^5 cells per well). Infected DCs (24 h.p.i.) were added at the
required concentration so that the DC:T-cell ratios ranged from 0 to 0.1. Five
days later, the cells were stained with a mouse anti-human CD3 antibody
(UCHT1; BD Pharmingen), followed by an Alexa 647 F(ab')2 fragment of goat
anti-mouse immunoglobulin G (Molecular Probes, United Kingdom). Analysis
was performed by flow cytometry using a FACS Calibur cytometer to detect the
decrease in CFSE fluorescence intensity resulting from cellular divisions.

**Statistical analysis.** Wilcoxon rank tests or paired Student’s t tests (in the case
of normal distribution) were used to determine statistical differences, using the
SigmaStat and R software.

**RESULTS**

DCs infected with *B. suis* do not engage their maturation
process. *Brucella* efficiently infects immature human DCs and
is able to grow extensively within these cells (5). In order to
determine the consequences for DC physiology, we analyzed the
expression of characteristic surface markers on immature
and *Brucella*-infected DCs. *E. coli*-infected DCs were used as a
positive control, since this bacterium induces strong matura-
tion of DCs (47). *Brucella*-infected cells maintained a DC phe-
notype, as shown by the stable level of expression of DC-SIGN
and CD1a (Fig. 1) and the absence of CD14 expression,
indicating that the bacteria did not cause the DCs to retreat
towards a monocyte/macrophage phenotype. Evident up-regu-
lation of membrane determinants involved in antigen presen-
tation (major histocompatibility complex classes I and II) and
costimulation (CD83, CD40, and CD86) were observed in *E.
coli*-infected DCs. In contrast, in *Brucella*-infected DCs, the
expression of these maturation markers was weakly modulated
compared to the expression in immature DCs. The slight in-
crease in CCR7 and CD83 expression meant that infection with
*Brucella* resulted in very limited activation of DCs, which
did not lead to cell maturation. Repeated experiments with
DCs from several donors (n = 22) confirmed that *Brucella-
infected cells did not exhibit increased expression of the che-
mokine receptor CCR7, of the antigen presentation molecules
HLA-ABC and HLA-D, and of the costimulation receptors
CD40, CD86, and CD83 (*P* < 0.001 for these six markers
compared to *E. coli*-infected DCs). Taken together, these
results showed that, in spite of a high infection rate and extensive
proliferation within DCs (involving close contact with a great
number of bacteria) (5), *Brucella* did not induce up-regula-
tion of maturation marker expression in human DCs.

*Brucella*-infected DCs are poor inducers of human naïve
T-lymphocyte proliferation. The functional impact of the in-
fection was assessed by analyzing antigen presentation activity of
*Brucella*-infected DCs to naïve T cells. Immature DCs were
infected with *Brucella* and *E. coli* as a positive control for
antigen presentation by fully mature DCs. After 24 h of infec-
tion, DCs were put in contact with allogeneic human naïve
CD45RA-CD4+CD45RA+ T cells intracellularly stained with CFSE and
plated at DC/T-cell ratios varying from 0.1 to 0.005. T-lymphy-
tocyte proliferation was determined 5 days later by flow cytom-
etry analysis of the decrease in CFSE (Fig. 2). DCs infected with
*E. coli* clearly showed a higher capacity to induce the
response of naïve T cells than immature resting DCs. In con-
trast, *Brucella*-infected DCs were unable to provide more ef-
ficient stimulation of T-cell proliferation than immature DCs,
and there was no statistical difference between the antigen-
presenting cell (APC) activity of immature DCs and that of
*Brucella*-infected DCs. This indicated that *Brucella* was also
able to circumvent DC maturation at the functional level.

**DCs infected with *Brucella* are poor producers of TNF-α.**

TNF-α is a multipotent inflammatory cytokine fundamental
for defense against a variety of intracellular pathogens and
plays an important role in DC maturation (44). Moreover, we
showed several years ago that *B. suis* specifically prevents
human macrophages from secreting TNF-α (5). Therefore, we
measured TNF-α secretion by human DCs infected with *Bru-
cella* or *E. coli* (Fig. 3). In contrast to the results obtained with
*E. coli*, which induced remarkable secretion of TNF-α, DC
invasion by *Brucella* did not promote any comparable produc-
tion of this cytokine. The concentrations of TNF-α in *Brucella-
infected DC supernatants remained very low and were just
above those observed in immature DC supernatants. The ab-
seence of IL-10 production by infected DCs (data not shown)
suggested that *Brucella* could affect the production of TNF-α in
an IL-10-independent way.

![FIG. 2. Ability of infected DCs to induce naïve T-lymphocyte pro-
liferation. At 24 h.p.i. DCs that were not infected (NI) or were infected
with *B. suis* or *E. coli* were tested for the ability to stimulate allogeneic
naïve CD4+ T lymphocyte proliferation as characterized by CFSE analysis
after 5 days of coculture. The data are means ± standard
errors of the means of 11 independent experiments. Statistical differ-
ences for comparisons with noninfected DCs, as determined by paired
Student’s t tests, are indicated as follows: one asterisk, *P* < 0.05; two
asterisks, *P* < 0.01; and three asterisks, *P* < 0.001.](http://iai.asm.org/Downloaded from)
Exogenous TNF-α promotes the maturation of Brucella-infected DCs and antigen presentation. Addition of E. coli lipo-polysaccharide (LPS) to Brucella-infected DCs led to complete phenotypic maturation of these cells (data not shown). This result implied that the lack of DC maturation during Brucella infection did not result from a total blockade of their engagement in maturation processes but more likely resulted from impairment of one or several parameters controlling these processes. Given that TNF-α is strongly implicated in DC maturation and that TNF-α knockout DCs fail to mature (44), the impairment of TNF-α secretion during DC infection could be a pertinent way for Brucella to avoid the maturation of infected cells. We analyzed the behavior of Brucella-infected DCs in the presence of exogenous TNF-α. DCs were infected with the bacteria, rhTNF-α was added at 16 h p.i. (the time of peak TNF-α secretion observed in E. coli-infected DCs [Fig. 3A]), and DC maturation was analyzed at 48 h p.i. (Fig. 4). DCs infected with Brucella and treated with exogenous TNF-α displayed highly significant enhancement of CCR7 and CD83 expression compared to untreated infected cells. Similarly, the expression levels of CD40, CD86, HLA-ABC, and HLA-D were up-regulated in the presence of TNF-α. Exogenous TNF-α did not increase expression of the maturation markers to the levels resulting from infection with E. coli. Nevertheless, TNF-α treatment undoubtedly promoted the initiation of maturation processes in Brucella-infected DCs.

In the presence of exogenous rhTNF-α, Brucella-infected DCs acquired the ability to induce powerful proliferation of naïve T cells that was quantitatively similar to that induced by fully mature E. coli-infected DCs (Fig. 5). These results indi-
cate that the impairment of TNF-α production observed upon Brucella infection could account for the restriction of DC maturation and for the absence of antigen presentation to naïve T lymphocytes.

The results described above also suggest that the amount of TNF-α produced upon E. coli infection was much greater than the amount required for the establishment of optimal APC activities. This was confirmed by biological assays (9); the TNF-α biological activity measured in culture supernatants of E. coli-infected DCs was at least 10-fold higher than the rhTNF-α biological activity allowing optimal APC activity of infected DCs (data not shown).

**omp25 and bvrR mutants of B. suis have reduced ability to control TNF-α secretion during human DC infection.** Omp25 is an outer membrane protein present on the surface of virulent Brucella, and Omp25-defective mutants are attenuated in vivo (14). Some years ago we demonstrated that this protein promoted the inhibition of TNF-α production following human macrophage infection with Brucella or stimulation of human macrophages by E. coli LPS (9, 27). Omp25 is also absent from the external membrane of Brucella with a mutation in the bvr operon (22), since the BvrRS two-component system regulates the expression of the omp25 gene. bvrR mutants of Brucella are attenuated in mice and proliferate neither in isolated macrophages (30, 52) nor in DCs (5). These findings prompted us to analyze the interactions of two mutants with human DCs in order to explore the relationship between the control of TNF-α secretion and the maturation of infected DCs.

Figure 6A shows the behavior of B. suis bvrR and omp25 mutants within DCs. At the onset of the experiments, the numbers of intracellular viable bacteria were similar for all bacterial strains. In accordance with our previous findings (5), the bvrR mutant did not proliferate within DCs. In contrast, the omp25 mutant did not display any significant impairment of intracellular proliferation compared to the WT, a result similar to that obtained in vitro with human macrophages (27).

When cytokine production was examined, human DCs infected with the omp25 or bvrR mutant produced more TNF-α than WT-infected DCs (for the WT-omp25 mutant comparison, P = 0.0363; for the WT-bvrR mutant comparison, P = 0.0014) (Fig. 6B), and the secretion was more pronounced with the B. suis bvrR mutant. The secretion of TNF-α induced by the omp25 mutant was similar to that previously measured with human macrophages (27). These findings indicated that, as in macrophages, Omp25 did not contribute to the intracellular proliferation of the bacteria but had an important role in the control of TNF-α production by infected DCs, with possible consequences for DC maturation.

**DCs infected with B. suis mutants mature and stimulate naïve T lymphocytes by a TNF-α-dependent mechanism.** Figure 7 shows the maturation of DCs infected with the omp25 and bvrR mutants of B. suis. The percentages of DCs expressing CCR7 and CD83 were higher in DCs infected with the mutants than in WT-infected cells. In the same way, the expression levels of CD40, CD86, and HLA-ABC were higher when infections were performed with mutants than when infections were carried out with the WT strain. This indicated that DCs infected with the omp25 or bvrR mutant of B. suis displayed significant phenotypic maturation compared to WT-infected cells.

However, the maturation level reached upon infection with the omp25 mutant was not equivalent to that observed in bvrR mutant-infected cells. Although the expression of CD40, CD86, and HLA-ABC was modulated equivalently in DCs infected with the two mutants, HLA-D expression was significantly induced in bvrR mutant-infected DCs (P = 0.03 for a...
comparison with the WT) but not in *B. suis* *omp*25 mutant-infected DCs despite a slight increase (*P* = 0.445 for a comparison with the WT). Moreover, CCR7 and CD83 were more strongly expressed in *B. suis* *bvrR* mutant-infected DCs than in *B. suis* *omp*25 mutant-infected cells (for CCR7, *P* = 0.005, for CD83, *P* = 0.039). This meant that the commitment to maturation processes induced with the *B. suis* *bvrR* mutant was deeper than the commitment induced with the *omp*25 mutant.

For each of the two mutants, the expression of CCR7 and HLA-ABC was equivalent to that measured in *B. suis*-infected cells treated with rhTNF-α (WT plus TNF-α [Fig. 7]). The same observation was made for HLA-D expression on *bvrR* mutant-infected DCs (but not on *omp*25 mutant-infected DCs). The CD83 and CD40 expression levels appeared to be slightly lower but still close to those measured in *B. suis*-infected DCs treated with rhTNF-α. Only CD86 expression was less potently up-regulated in mutant-infected cells than in WT-infected rhTNF-α-treated DCs.

This phenotypic analysis was confirmed by assessment of the stimulating capacities of mutant-infected DCs. Figure 8A shows the proliferation of naïve T cells cultured for 5 days in the presence of DCs infected with the *B. suis* *omp*25 or *bvrR* mutant at a DC/T-cell ratio of 0.025 (1:40). Upon infection with these mutants, DCs acquired the capacity to efficiently present antigen to naïve T cells. Again, the effect was more pronounced with the *bvrR* mutant than with the *omp*25 mutant (*P* = 0.01); the former displayed approximately 80% of the stimulation obtained with *E. coli*, whereas the latter displayed only about 50% of the full-stimulation effect.

Thus, upon infection by Omp25-defective mutants, DCs matured and acquired the ability to present antigens, and these effects were stronger when the lack of Omp25 protein was associated with impairment of the BvrR/S system. In order to determine whether TNF-α secreted during DC infection with mutants is responsible for the induction of DC maturation, the experiments described above were repeated in the presence of anti-TNF-α blocking antibodies. When these antibodies were added at the onset of infection, the *omp*25 and *bvrR* mutants were unable to induce an increase in maturation marker expression (data not shown). In parallel, mutant-infected DCs failed to stimulate naïve T cells. Figure 8B shows the proliferation of naïve T cells cultured with *omp*25 or *bvrR* mutant-infected DCs with or without anti-TNF-α blocking antibodies. For each mutant, the inhibition of TNF-α biological activity led to a complete collapse of antigen presentation activities (*P* < 0.001 for both mutants), which fell to the basal level measured in DCs infected with virulent *Brucella* or in immature uninfected DCs (Fig. 8B).
the secretion of cytokines involved in the polarization of the immune response, we measured IL-12 concentrations in culture supernatants of DCs infected with WT or attenuated strains (Fig. 9). DCs infected with virulent *B. suis* produced smaller amounts of IL-12 than cells infected with the *omp25* or *bvrR* mutant (*P* < 0.05 and *P* < 0.01, respectively, calculated from seven independent experiments). Infection with the *bvrR* mutant of *B. suis* led to more intense IL-12 secretion (*P* = 0.031 for a comparison of the *bvrR* and *omp25* mutants [n = 7]). These results suggested that infection with these mutants not only induced DC maturation but could also favor Th1 polarization of the adaptive response.

**DISCUSSION**

We have recently established that, unlike most intracellular bacteria, *Brucella* invades human DCs and grows extensively within them. In order to assess whether this behavior of *Brucella* specifically alters DC functions, we analyzed the consequences of *Brucella* infection for DC physiology and characterized the implications of some virulence factors. Unlike monocyte-derived DCs infected with *Mycobacterium* (35), *Brucella*-infected DCs retained a DC phenotype, as shown by DC-SIGN expression and the absence of CD14. A dedifferentiation of infected DCs towards a monocyte-macrophage phenotype would obviously have had dramatic consequences for immune response initiation. However, even though the differentiation state of human DCs was not modified, infection with *Brucella* did not trigger DC maturation, as demonstrated by the low level of expression of adequate markers on the cell surface and by the inability of infected cells to present antigen to naïve T cells. A comparable inhibition of DC maturation was reported previously after in vitro infection with various intracellular pathogens, notably several viruses, including herpes simplex virus, hepatitis C virus, vaccinia virus, human T-cell leukemia virus, and measles virus (33), and also intracellular parasites, including *Toxoplasma* and *Trypanosoma* (45). In contrast, the majority of intracellular bacteria induce DC maturation during their interaction with these cells; this is particularly true for *Bordetella* (16), *Listeria* (42), *Chlamydia* (40), and *Salmonella* (38, 53). For *Mycobacterium tuberculosis*, although a study reported inhibition of *Mycobacterium*-infected DC maturation (23), several other workers have observed maturation of human and murine infected DCs (11, 24). Fewer reports are available about the interaction between *Legionella* and DCs, and the induction of DC maturation by these bacteria still seems to be debated (28, 36, 37). Of the two
bacteria which, like Brucella, proliferate in human DCs, Francisella apparently fails to control maturation and seems to make DCs develop towards an aberrant activation state (4, 6), whereas Coxiella manages to avoid DC maturation owing to its low endotoxic properties (48). Brucella compounds are also reported to be weakly endotoxic and participate in the stealthiness of the bacteria (25, 32). However, the low stimulatory activity of Brucella cannot explain by itself the lack of DC maturation since Omp25-defective bacteria did induce significant maturation in spite of their endotoxic activity comparable to that of WT Brucella (34). Therefore, the unusual absence of DC maturation during interaction with WT Brucella involves mechanisms in which TNF-α seems to play a key role: (i) TNF-α, which is an essential factor for DC maturation (44, 54), was not produced during DC infection; (ii) TNF-α addition to the infected DCs overcame the lack of both phenotypic and functional maturation of the cells; (iii) mutant-infected DCs, which produced TNF-α, displayed characteristics of maturation; and (iv) blocking anti-TNF-α antibodies prevented the maturation of omp25 and bvrR mutant-infected DCs. Our results thus define an original virulence strategy by which Brucella could manipulate the specific immune response by targeting DC functions.

In contrast to WT Brucella, the two Omp25-defective mutants induced TNF-α production by infected DCs. This observation agrees with previous data obtained with macrophages (27) and suggests that TNF-α secretion is probably similarly regulated in human DCs and macrophages. It also indicates that impairment of TNF-α production is not required for the intracellular development of Brucella. Therefore, the decrease in TNF-α production is relevant only to the developing immune response and inflammation.

As previously reported for macrophages, the Omp25 protein does not definitively block TNF-α secretion, which remains inducible (9, 27), Brucella is thus able to prevent DC maturation but does not strictly impair it, as shown by treatment of infected cells with rHTNF-α (or with E. coli LPS). However, whether the low level of TNF-α secretion is the only parameter accounting for the weak maturation of infected DCs remains unclear; we cannot prejudge eventual Omp25-mediated complementary mechanisms not related to TNF-α control that could also down-regulate infected DC maturation. Nevertheless, the abrogation of TNF-α biological activity with blocking antibodies is sufficient to suppress the maturation of mutant-infected DCs, proving the key role of this cytokine in the maturation avoidance strategy.

The possible contribution of complementary mechanisms is supported by the less intense maturation of omp25 mutant-infected DCs than of bvrR mutant-infected DCs. This could result directly from the very different fates of the two mutants within their host cells. Actually, the intracellular proliferative capacity of the B. suis omp25 mutant could allow persistent virulence processes, while the elimination of the intracellular bvr mutant could lead to more potent stimulation of DCs by degraded bacterial compounds. We cannot exclude the possible direct implication of other bacterial factors regulated by the BvrRS two-component system. Indeed, bvrR invalidation alters the expression of more than 100 proteins of Brucella, including Omp3 family proteins, peptidoglycan-related proteins, and several lipoproteins (31). Moreover, if lipid A undecacylation in the bvrR mutant does not affect LPS endotoxic activities (34), it could lead to exposition of molecules then recognized by DCs. All of these phenomena could act together with Omp25 to strengthen the control of TNF-α secretion, explaining the larger amount of this cytokine produced upon infection with bacteria lacking both the BvrR and Omp25 proteins than upon infection with the omp25 single mutant. These factors could play a role in another mechanism implicated in the restraining of DC maturation complementary to the main strategy described here involving the control of TNF-α secretion.

Until now, the drastic attenuation of Omp25-defective Brucella in vivo was attributed to the involvement of this protein in the impairment of TNF-α secretion in macrophages (27), whereas the attenuation of bvr mutants was related to their inability to proliferate within macrophages (52). Indeed, we show here that, due to their inability to control TNF-α secretion, Omp25-defective bacteria fail to dampen DC maturation and consequently authorize the establishment of a specific immune response, independently of bacterial intracellular proliferation. All of these results could explain why Omp25-defective mutants are attenuated in vivo and why they have been reported to induce protection at least equivalent to that induced by reference vaccine strains (14). In a vaccinal context, the weaker capacity of omp25 mutants to induce adaptive immunity could be compensated for by the duration of maturation signals resulting from their efficient proliferation during the first phase of host infection (14, 15).

Persistent infection with Brucella is associated with a decrease in the capacity for T-lymphocyte proliferation (19) and with down-activation of CD4+ T cells, particularly during relapses of chronic infection (50). Antibiotic treatment leads to normalization of these parameters, confirming the connection between the persistence of living bacteria within patients and the reported defect in a T-cell response. Thus, an association can be proposed between the impairment of maturation and APC activities of DCs by Brucella, and the immunological status of brucellosis patients. A Th2-oriented response is usually observed in chronic brucellosis patients (19, 43), whereas protection against Brucella is conferred by a Th1-polarized response. Myeloid DCs have been implicated in the induction of Th1 responses, particularly through IL-12 secretion. The absence of both IL-12 production and T-cell stimulatory activity in Brucella-infected DCs clearly suggests that by altering and preventing myeloid DC functions Brucella specifically avoids the development of a protective Th1 immune response. Moreover, human immature DCs interacting with naïve CD4+ T cells could induce a regulatory T-cell response and inhibit the Th1 response (17). By contact with Brucella-infected immature DCs, CD4+ naïve T cells could thus develop in Brucella-specific regulatory T cells, which could then delay the adaptive immune response. Furthermore, due to their inefficient interaction with Brucella-infected DCs, CD4+ T cells could fail to provide the licensing signals required for subsequent stimulation of CD8+ cytotoxic T cells by infected DCs (51).

A recent publication suggested that a peculiar DC subpopulation, called inflammatory DCs, could participate in the effector phase of the anti-Brucella immune response in infected mice (12). Nevertheless, host interactions with Brucella, as well
as the subpopulations of DCs, are not fully equivalent in mice and humans. Therefore, it is still possible that such a mechanism cannot be transposable to humans.

To summarize, our data show that *Brucella* avoids maturation and APC activities of human myeloid DCs through Omp25-dependent mechanisms disrupting TNF-α secretion by infected cells. The crucial importance of TNF-α in this bacterial escape strategy enlightens previous reports on the association of chronic *Brucella* infections with an unfavorable TNF-α promoter polymorphism (8, 13). Altogether, the data suggest that some host genetic factors could confer a particular susceptibility to chronic brucellosis and be decisive for spontaneous healing or establishment of chronic disease by impeding the complete eradication of the pathogens. In conclusion, targeting DC maturation through TNF-α regulation could be an essential parameter of the *Brucella* virulence strategy and pathogenicity.

ACKNOWLEDGMENTS

This work was supported by institutional grants from INSERM. We thank Sejal Morjaria for attentively correcting and improving the manuscript.

REFERENCES


Downloaded from http://iai.asm.org/ on October 31, 2017 by guest
that facilitate selection of antigens for MHC class II presentation. Immunity 18:813–823.


Editor: D. L. Burns