Kafkas Univ Vet Fak Derg
26 (6): 749-755, 2020
DOI: 10.9775/kvfd.2020.24280

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

The Deletion of *Omp19* Gene of *Brucella abortus* 2308 Reduces Its Survival in Mouse Macrophage and in Mice

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Article ID: KVFD-2020-24280 Received: 01.04.2020 Accepted: 30.07.2020 Published Online: 30.07.2020

How to Cite This Article

Wang Y, Ma Z, Zhang H, Yi J, Wang Y, LiT, Chen C: The deletion of *Omp19* gene of *Brucella abortus* 2308 reduces its survival in mouse macrophage and in mice. *Kafkas Univ Vet Fak Derg,* 26 (6): 749-755, 2020. DOI: 10.9775/kvfd.2020.24280

Abstract

The aim of this study is to investigate the role of outer membrane protein *Omp19* of Brucella in its pathogenesis. In this research, the *Brucella* 2308 Δ*Omp19* deletion mutant was constructed and its intracellular survivability was evaluated in murine macrophages RAW264.7 and BALB/c mice. We also analyzed the lysosomal fusion induced by and cytotoxicity of Brucella in murine macrophages RAW264.7. The results showed that the *Brucella* 2308 Δ*Omp19* induced higher levels of immunoprotective cytokines *in vitro* than *Brucella* 2308, and the number of intracellular bacteria in RAW264.7 macrophages was lower after *Brucella* 2308 Δ*Omp19* infection than after *Brucella* 2308 infection. *In vivo, Brucella* 2308 Δ*Omp19* induced protective immune response in mice, and the splenic bacterial load of this deletion mutant was lower than that of *Brucella* 2308. In addition, the Brucella 2308 Δ*Omp19* also promoted the fusion of *Brucella*-containing vacuoles and lysosomes, in macrophages. Therefore, *Brucella* 2308 Δ*Omp19* reduced virulence compared with *Brucella* 2308 and activate the immune response of the host, and is a promising candidate for a live attenuated vaccine.

Keywords: *Brucella, Omp19 gene, Homologous recombination, Viability, Intracellular survival*

Brucella abortus 2308'in *Omp19* Geninin Silinmesi Faregil Makrofajında ve Farelerdeki Sağkalımını Azaltır

Öz

Bu çalışmanın amacı *Brucella*'nın dış zar proteini *Omp19*'un bakterinin patogenezindeki rolünü araştırmaktır. Bu araştırmada, *Brucella* 2308 Δ*Omp19* delesyon mutantı oluşturularak faregil makrofajları RAW264.7 ve BALB/c farelerindeki hücre içi canlılığı değerlendirildi. Ayrıca faregil makrofajları RAW264.7'de *Brucella*'nın neden olduğu lizozomal füzyonu ve sitotoksisite de analiz edildi. Sonuçlar, *Brucella* 2308 Δ*Omp19*'un *in vitro* olarak immünoprotektif sitokin üretimini *Brucella* 2308'den daha fazla uyardığını ve RAW264.7 makrofajlarındaki hücre içi bakteri sayısının *Brucella* 2308 Δ*Omp19* enfeksiyonunda *Brucella* 2308 enfeksiyonundan daha düşük olduğunu gösterdi. *In vivo* olarak, *Brucella* 2308 Δ*Omp19*, farelerde koruyucu bağışıklık tepkisine neden oldu ve bu delesyon mutantının dalak bakteri yükü, *Brucella* 2308'den daha düşüktü. Bunun yanı sıra *Brucella* 2308 Δ*Omp19*, makrofajlarda Brucella içeren vakuollerin ve lizozomların füzyonunu da uyardı. *Brucella* 2308 Δ*Omp19*, *Brucella* 2308 ile karşılaştırıldığında düşük virulansla konağın bağışıklık tepkisini aktive etmiştir ve zayıflatılmış canlı aşı için umut verici bir adaydır.

Anahtar sözcükler: *Brucella, Omp19 geni, Homolog rekombinasyon, Canlılık, Hücre içi sağkalım*

INTRODUCTION

Brucellosis is a zoonotic chronic infectious disease caused by the members of bacterial genus *Brucella*, which infects almost all mammals, causing disease [1]. *Brucella* infection can lead to abortion in pregnant livestock, orchitis in male animals, etc. The main manifestations of infection in humans are fluctuating fever, muscle soreness, and joint swelling and pain [2,3]. However, except for some antibiotic and corticosteroids, there is no safe and effective *Brucella* medicine for humans or animals, so a new treatment method that provides high-level protection is urgently required.

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The main outer membrane proteins (OMPs) of *Brucella* include Omp10, Omp16, *Omp19*, SP41 and BepC, which are closely associated with the virulence of *Brucella* in previous study [4-6]. The molecular weight of *OMP19* is about 19 kDa. and it plays an important role in the structure of the outer membrane and bridges the outer membrane protein of *Brucella* and polymyxin [7,8]. Recent research shows that *Omp19* and Omp10 do not change their virulence and OM properties in *Brucella ovis* PA mutants, but they have an interchangeable function to maintain the bacterial outer membrane integrity [9]. Moreover, *Omp19* could enable *B. abortus* to evade the antimicrobial activity of proteolytic defense system in host [5]. Several studies have reported that the OMP10 mutant (ΔOmp10) survives less well in infected mice than its parental strain [7,8]. Similar to ΔOmp10, Δ*Omp19* mutant highly weaken virulence in mouse infected by oral route [5]. However, the detailed functions and mechanisms of *Omp19* in host is unknown.

Brucella survives and proliferates in specialized unprofessional phagocytes in animals [10], mainly parasitizing macrophages, dendritic cells, and embryonic trophoblast cells. It invades the cell early, in the form of a membrane-bound vesicle in *Brucella*-containing vacuoles (BCVs). The maturation of BCVs in the mid stage of infection inhibits the binding of lysosomes to *Brucella*, allowing it to escape the killing mechanism of the host cell. The interaction between BCVs and the secretory trafficking of host cell in the later stage of infection causes the BCVs to fuse to the endoplasmic reticulum, triggering autophagy, and facilitating the long-term parasitism and proliferation of *Brucella* [2,11]. *Brucella* also inhibits cell apoptosis and the host immune response by producing different virulence factors, thus promoting its own intracellular survival [12,13]. This study aimed to explore the function of the outer membrane protein *Omp19* of *Brucella*, and tried to clarify its important role in the pathogenesis of *Brucella*, provide a reference for the research and development of *Brucella* candidate vaccine.

MATERIAL and METHODS

Ethical Approval

All animals used were treated humanely and in accordance with institutional animal care guidelines in our experiment. This study was approved by the Animal Care and Use Committee of Shihezi University.

Strains, Cells, and Laboratory Animals

Brucella abortus strain 2308 was provided by the China Center for Disease Control and Prevention (Beijing, China) and cultured in tryptone soya agar (TSA) or tryptone soya broth (TSB) (Oxoid, England). *Escherichia coli* strain DH5α was cultured in Luria–Bertani medium (Difco, Becton Dickinson). The pGEM-7Zf+ plasmid was purchased from Promega Corporation (Madison, WI, USA). Mouse RAW264.7 cells were purchased from the Cell Resource Center, the Institute of Basic Medical Sciences of the Chinese Academy of Medical Sciences/Peking Union Medical College (Beijing, China), and cultured in Dulbecco's modified Eagle's medium (Gibco, USA) containing 10% calf serum (Gibco) at 37°C under 5% CO_2. A total of 75 six-week-old female BABL/c mice were provided by the Experimental Animal Center of the Academy of Military Medical Science (Beijing, China). We randomly divided them into two groups (a part of 60 mice, a part of 15 mice), and each group was divided into three subgroups (*Brucella* 2308, *Brucella* 2308 Δ*Omp19* and PBS). All experimental procedures and animal care protocols were performed in compliance with Institutional Animal Care Regulations.

Construction of Brucella 2308 Omp19 Gene Deletion Strain

The upstream and downstream homologous primers for the *Omp19* gene were designed with Primer 5.0, based on the gene sequence of international standard strain 2308 of *B. abortus*, published in GenBank. The upstream primers were *Omp19*-N-F GGATCCCGATAAACAGCGTCGGATAGC (*Bam*HI) and *Omp19*-N-R GGGCATGGAACCTCTCTCTGC GGAAACGAGAGAGATAC, and the downstream primers were *Omp19*-C-F CAGTTCTCCATTTGCGC and *Omp19*-C-R GAGCTCGCAGTCTATCGTGTCGGA (*Sac*I). The upstream and downstream target fragments were recovered, amplified with fusion PCR, and then linked to the pMD18-T Simple (TaKaRa, Japan) vector. After screening and sequencing, positive clones of pMD18-T-*Omp19* were obtained. The recombinant plasmid pMD18-*Omp19* was identified by double digestion with *Bam*HI and *Sac*I. The target fragment was linked to the suicide vector pGEM-7zf+. The *sacB* gene is present in *Bacillus subtilis* and can be used as a selective marker for *Brucella*. The *sacB* gene was amplified with primers *SacB*-F GAGCTCGGGGAAGGAAGCACCGCTA (*Sac*I) and *SacB*-R GAGCTCGCTTATTGTTAATTGTCC (*Sac*I). *Sac*I restriction endonuclease was used to digest pGEM-7zf+-*Omp19* and the *sacB* gene, and the target fragments were ligated with T4 ligase. The recombinant homologous suicide vector pGEM-7zf+–*Omp19*–*SacB* was constructed by connecting EM-7zf+-*Omp19* to the *sacB* fragment. Competent *Brucella* 2308 cells were then transformed with the suicide vector using electroporation. Positive clones were screened according to their antimicrobial resistance. Primers *Omp19*-test-FGTCCGCAATGTCGTCAC and *Omp19*-test-R TCCATTCTTCGGCT were used to amplify the positive clones many times until positive clones were isolated.

Determining the Intracellular Viability of Brucella 2308 ΔOmp19 in RAW264.7 Cells

Mouse macrophages were subcultured in six-well plates. *Brucella* 2308 and 2308 Δ*Omp19* were cultured to logarithmic phase. The cells were infected with *Brucella* in a bacterium: macrophage ratio of 100:1. After infection for 1 h, 2.5 μL of gentamicin (50 μg/mL) was added to each well of the six-well plate for 50 min to kill any extracellular *Brucella*. After

4, 12, 24, and 48 h, the cells were collected and counted. Lysozyme (0.2%) was added to release the intracellular bacteria, and the bacterial was diluted 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}, and then used to solid *Brucella* culture medium. The bacteria were cultured in an inverted incubator at 37°C for 3-4 days. The numbers of bacteria in the dishes were recorded.

Measuring Attenuation of Brucella 2308 ΔOmp19 Virulence in BALB/c Mice

To detect the effect of *Brucella* 2308 Δ*Omp19* on the survival of bacteria in mice, the *Brucella* 2308 and *Brucella* 2308 Δ*Omp19* strains were cultured to logarithmic phase and diluted to $5.0x10^6$ CFU/mL. An aliquot (0.2 mL) was intraperitoneally injected into BALB/c mice ($1.0x10^6$ CFU/mouse). The mice in the blank control group were injected with 0.2 mL of phosphate-buffered saline (PBS). There, we used 60 mice which was divided into three groups (*Brucella* 2308, *Brucella* 2308 Δ*Omp19* and PBS). Five mice were selected from each group on days 3, 7, 14, and 28 after injected strains. The mice were killed with CO_2 and their spleens were weighed under sterile conditions. The homogenates were diluted and coated on solid *Brucella* culture medium. The average colony-forming units (CFU) for each group was calculated after 3-5 days.

Cell Cytotoxicity Assay

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme, and the amount of LDH released can be used to calculate the numbers of dead and damaged cells. Macrophages were added to 6 six-well plates and infected with *Brucella* 2308 or the *Brucella* 2308 Δ*Omp19* mutant strain at a multiplicity of infection (MOI) of 10, 100, or 1000. At different times post infection, the supernatant was collected, and the levels of LDH released into it were measured with a Cytotoxicity LDH Detection Kit.

Effect on Lysosomal Fusion

The *Brucella* 2308 and *Brucella* 2308 Δ*Omp19* strains were used to infect mouse macrophages RAW264.7 in a ratio of 200:1. Lysosome staining reagent (250 μL/well) was added to the macrophages after 4, 24, or 48 h, and the cells were incubated at 37°C for 2 h. Paraformaldehyde (4%; 1 mL/well) was added after 30 min, and 0.3% Tranton X-100 (1 mL/well) was added for 10 min to cause cell permeation. Bovine serum albumin (1 mL/well) was added and the cells were incubated for 20 min. A sheep anti-*Brucella* IgG primary antibody (0.5%, 1 mL) was added and the cells were incubated overnight at 4°C. The primary antibody was removed, and 1 mL of a tetramethylrhodamine (TRITC)-labeled donkey anti-sheep IgG secondary antibody (0.2%) was added and the cells were incubated at 37°C for 1 h. The secondary antibody was removed and the cells washed with distilled water and sealed with 50% glycerol. The localization of *Brucella* and the lysosomes was observed in RAW264.7 cell samples under confocal microscopy after post infection 4 h, 24 h and 48 h, and the images were saved. The red spots were TRITC-labeled *Brucella* (BCVs), the green spots were fluorescein-isothiocyanate-labeled lysosomes, and the yellow spots were produced when the red and green signals merged in the figure, indicating the colocalization of BCVs and lysosomes. The fluorescent spots in each field of vision were recorded with a counter. The proportion of yellow signals among the red signals was calculated.

Cytokine Production Assay

After *Brucella* infection, we used an ELISA to evaluate the Cytokines levels in serum samples from mice or RAW264.7 cells. Fifteen mice were used here, also randomly divided into three groups. The cell culture medium from *Brucella*-infected cells (at 4, 12, 24, and 48 h) and serum samples from *Brucella*-infected mice (at 4, 12, 24, and 48 days) were obtained by *centrifugation* (3000 x g, 20 *min*). The interleukin 12 (IL-12) and tumor necrosis factor α (TNF-α) in the medium and the interferon γ (IFN-γ) in the serum samples were detected with enzyme-linked immunosorbent assays (ELISAs; ELISA Quantikine Mouse Kits, R&D Systems), according to the instructions of the manufacturer. For all chemokines, three parallel assays were performed, which were repeated at least three times.

Data Analysis

We looked for information which was benefited and determined the exact changes in the test group by comparing the differences between the test group and the control group. The experimental results for each group were analyzed with Scheffe tests, when finding a significant difference between the averages in each group, check whether there is a difference between one or more pairs of averages. In addition, we also used the One-way ANOVA test method. A P value of <0.05 or <0.01 was considered significant. All results are expressed as means ± SD. Data analysis We use IBM SPSS Statistics software.

RESULTS

Interaction Between BCVs and Lysosomes in Host Cells

The number of yellow spots, indicating the colocalization of the BCVs and lysosomes, was higher when the cells were infected with *Brucella* 2308 Δ*Omp19* than when they were infected with *Brucella* 2308 at 4-48 h. At 4 h, 100% of *Brucella* 2308 Δ*Omp19* (green) colocalized with lysosomes (red) and the patterns were similar at 24 and 48 h post infection (93% and 91%). These fusion rates were all significantly higher than the *Brucella* 2308-lysosome fusion rates at similar time points after infection ($P<0.05$; *Fig. 1*). The detailed data are shown in *Table 1*. Therefore, the knockdown of the *Omp19* gene of *Brucella* 2308 enhanced the fusion of BCVs and lysosomes.

Intracellular Survival of Brucella 2308 ΔOmp19 is Attenuated in Murine Macrophages (RAW264.7)

The intracellular survival of *Brucella* 2308 gradually increased from 4 h post infection. However, the survival of *Brucella* 2308 Δ*Omp19* gradually decreased from 12 h post infection. At 12 h after infection, the numbers of surviving *Brucella* 2308 Δ*Omp19* and *Brucella* 2308 strains differed (P<0.05). By 24 and 48 h after infection, the number of strain *Brucella* 2308 Δ*Omp19* bacteria was significantly lower in the host cells than the number of strain *Brucella* 2308 (P<0.01), the range of decrease by almost 50% of that of 2308 strains *(Fig. 2)*.

Brucella 2308 ΔOmp19 Induced High Levels of IL-12 and TNF-α Release from RAW264.7 Cells

The production of neither IL-12 nor TNF-α differed negligibly in the two strains at 12 h post infection, but at 24 h post infection, the difference was significant that *Brucella* 2308 Δ*Omp19* higher than *Brucella* 2308. The difference in IL-12 was significant that peaked at 24 h post infection, whereas TNF-α production peaked at 48 h post infection *(Fig. 3)*. Therefore, *Brucella* 2308 Δ*Omp19* increased the secretion of immune cytokines IL-12 and TNF-α, which are not conducive to the survival of *Brucella* in cells.

Reduced Virulence of Brucella 2308 ΔOmp19 in Mouse Model

The counts of *Brucella* 2308 Δ*Omp19* in the mouse spleens were significantly lower than those of *Brucella* 2308 on days 3, 7, 14, and 28 after infection (P<0.05; *Fig. 4)*. At 28 days post inoculation, *Brucella* 2308 Δ*Omp19* was virtually cleared from the spleens of the mice. All these results

Table 1. *Fusion rates of Brucella -containing vacuoles and lysosomes*

Strains	4 h	24 h	48 h
2308	10.00%	32.70%	73.20%
2308 ΔOmp19	100%	93.60%	91%

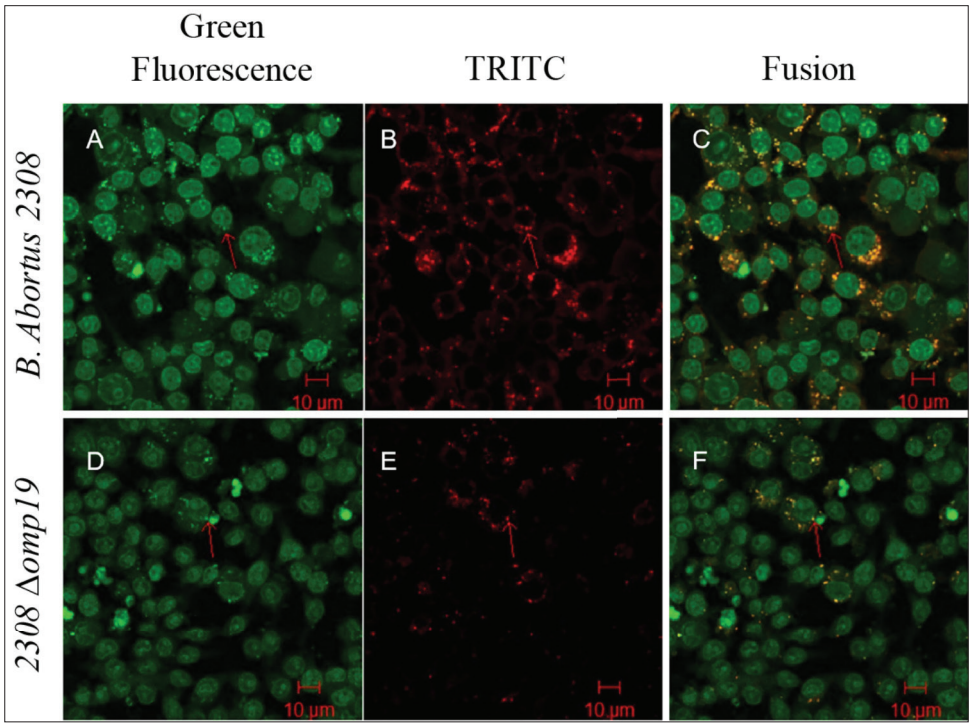


Fig 1. Interaction of *Brucella* 2308 or *Brucella* 2308 ΔOmp19 with RAW264.7 macrophage lysosomes. *Brucella* was labeled with a sheep anti-*B. abortus* IgG antibody and RITC-conjugated donkey anti-*B. abortus* IgG antibody, and lysosome was labeled with fluorescein-isothiocyanate to display green fluorescence. The fixed cells were examined at different times after infection, and the infected cells were imaged with confocal microscopy 4 h after infection. The fusion rate of phagosome-lysosome was calculated from the colocalization of the green fluorescent and TRD markers. The bacteria in each time period were analyzed to determine the fusion rate

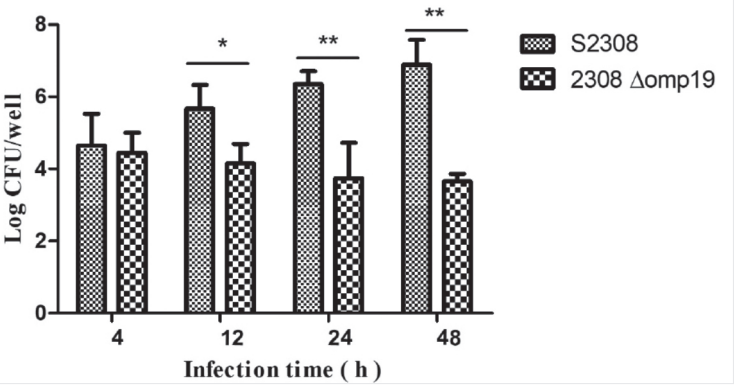


Fig 2. Intracellular viability analysis of *Brucella* 2308 and 2308 ΔOmp19 strains in RAW264.7 mouse macrophages. Macrophages were infected with *Brucella* 2308 or the *omp19*-deleted strain and incubated for specific times. The numbers of bacteria in the RAW264.7 cells were counted at different time points. Each point represents the mean ± standard deviation of three experimental groups. The number of bacteria in the RAW264.7 cells marked with an asterisks (* P<0.05, ** P<0.01) differed significantly in the parental and mutant strains

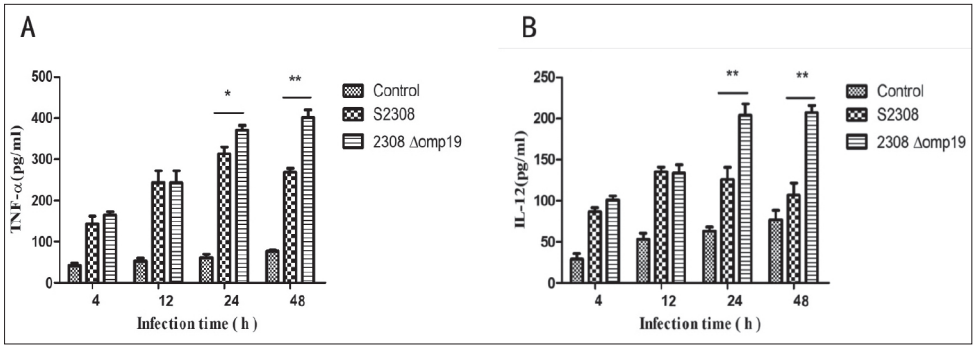
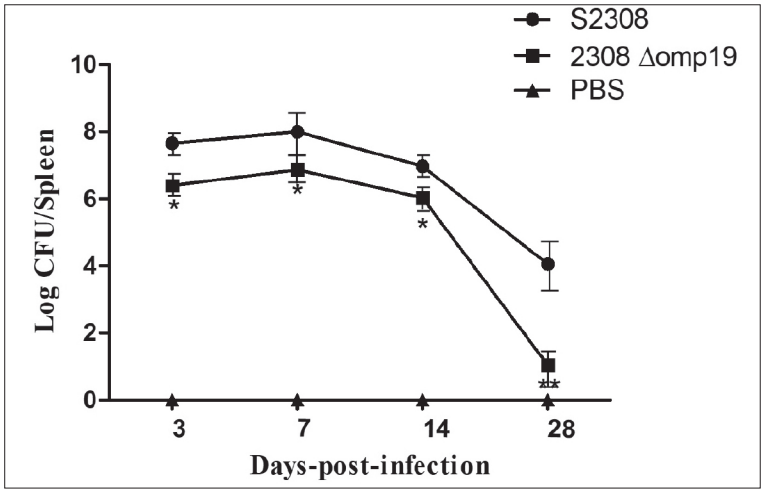


Fig 3. Levels of IL-12 and TNF-α released from RAW264.7 cells infected with *Brucella* 2308 or *Brucella* 2308 ΔOmp19. After bacterial infection, the release of IL-12 and TNF-α was measured at 4, 12, 24, and 48 h with ELISAs. Each point represented the mean ± standard deviation of three experimental groups. Asterisk (* P<0.05, ** P<0.01) indicate significant differences in the release of IL-12 and TNF-α between the parental and mutant strains after infection

Fig 4. Survival of *Brucella* 2308 and *Brucella* 2308 ΔOmp19 in the mouse model. *Brucella* 2308 or *Brucella* 2308 ΔOmp19 ($1x10^5$) was injected into the abdominal cavities of the mice in each group. Values are the averages ± SEM for 20 mice. Each point represents the mean ± standard deviation of three experimental groups. The Asterisks (* P<0.05, ** P<0.01) indicate significant differences in bacterial growth between the parental and mutant strains



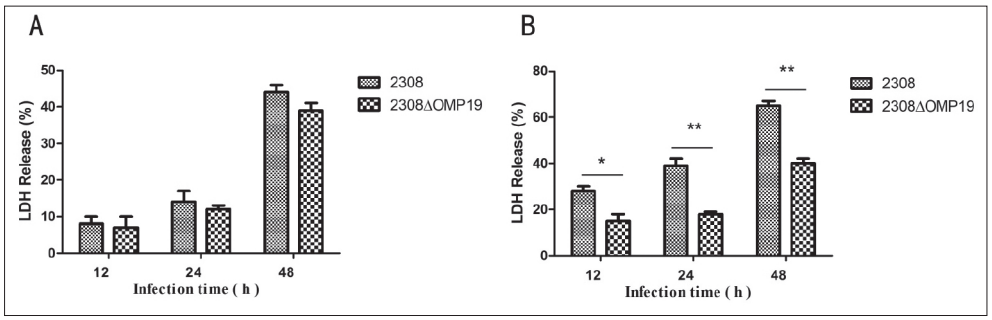


Fig 5. Levels of LDH released from cells infected with *Brucella* 2308 or *Brucella* 2308 ΔOmp19. LDH release was measured at 12, 24, and 48 h after *Brucella* infection. A. MOI = 10; B. MOI = 100. Asterisks (* P<0.05, ** P<0.01) indicate significant differences in LDH release between the parental and mutant strains

indicate that *Brucella* 2308 Δ*Omp19* is less virulent than its parental strain *(Brucella* 2308).

Omp19-Regulated Cytotoxicity of Brucella 2308 for Mouse Cells

To determine whether the cytotoxicity of *Brucella* 2308 for mouse cells is regulated by the *Omp19* protein, we measured the LDH released from mouse cells after *Brucella* 2308 or *Brucella* 2308 Δ*Omp19* infection at an MOI of 10, or 100. At 12, 24, and 48 h post infection, there were no significant differences in LDH release when the cells were infected with *Brucella* 2308 or its mutant at an MOI of 10 *(Fig. 5-A)*, whereas when the cells were infected at an MOI of 100, the LDH released by the differently infected cells differed significantly at 12 and 24 h (P<0.05; *Fig. 5-B)*. At an MOI of 100, *Brucella* 2308 induced high levels of LDH release at 12 and 48 h, but *Brucella* 2308 Δ*Omp19* induced negligible LDH release. Both strains induced high levels of LDH release after infection for 48 h. Therefore, as the MOI increased, the LDH release rate also increased. These results indicate that *Omp19* regulates the cytotoxicity of *Brucella* 2308 for macrophages.

Immune Cytokine Production in Mice after Infection with Brucella 2308 or Brucella 2308 ΔOmp19

The total expression of IFN-γ induced by both *Brucella* strains gradually increased with time relative to that in the PBS-treated control group, and peaked at 14 days post infection. The *Brucella*-2308-Δ*Omp19*-vaccinated mice produced significantly higher IFN-γ levels than the *Brucella*-2308-infected mice (P<0.05*; Fig. 6)* from day 7 to day 28. These results indicate that the *Brucella* 2308-Δ Δ*Omp19* strain induces a high humoral response, which may protect the host against wild-type *Brucella* infection.

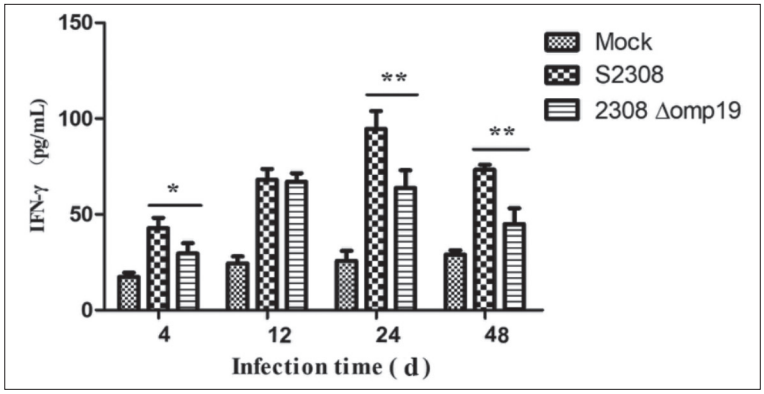


Fig 6. IFN-γ levels in mice infected with *Brucella* 2308 or the *Brucella* 2308 ΔOmp19 mutant. Each mouse was infected with 1x10^5 *Brucella* cells. Serum IFN-γ was measured at 4, 12, 24, and 48 days postinfection. Asterisks (* P<0.05, ** P<0.01) indicate significant differences in IFN-γ expression induced by the parental strain and mutant strain in mice

DISCUSSION

The intracellular survival of *Brucella* requires the participation of virulence factors, including outer membrane proteins, lipopolysaccharide (LPS), virB-encoded type IV secretion system, and virulence regulatory proteins. These virulence factors not only ensure the survival of the bacteria, but also kill the host cell. Vemulapalli et al.[14], reported that the *Brucella* protein BMP18 *(Omp19)* is one of its main virulence factors [14,15], and it is one of the most-studied outer membrane proteins. Silencing *Omp19* can reduce the bacterium's resistance to polymyxin B and deoxycholate [7] because the loss of the protein changes the inherent structure of the LPS in the outer membrane and disrupts the interactions of LPS with other molecules, thus altering the properties of the outer membrane [16]. However, knocking out the *Omp19* gene in *Brucella* RB51 did not alter its invasive capacity because *Brucella* RB51 is a rough vaccine strain in which LPS has no O-side chain [7]. In addition, *Omp19* protein indeed involved in cell invasion by *Brucella* and enhanced its intracellular survival [4,5], but clearly mechanism is unknown.

Therefore, in this study, we constructed an *Omp19* deletion mutant of *Brucella* 2308 and evaluated its virulence in cells and mice. The virulence of *Brucella* 2308 in cells was significantly reduced after the deletion of the *Omp19* gene, which may be attributable to an indirect effect of the deletion on the interaction between the outer membranes of *Brucella*, which would directly affect the invasion capacity and intracellular viability of *Brucella* 2308 Δ*Omp19*. The *cfu* counts of the *Brucella* 2308 Δ*Omp19* mutant in the spleens of mice were also lower than those of the virulent strain *Brucella* 2308, and the mutant was cleared faster than *Brucella* 2308. The ability of the spleen to carry *Brucella* can reflect the virulence of the bacterium, and we demonstrated that the *Omp19* gene of *Brucella* 2308 facilitates its invasion of its host cells and prolongs its intracellular survival. *Brucella* 2308 Δ*Omp19* is also a potential vaccine candidate.

Why is the survival of the *Brucella Omp19*-deleted strain in cells lower than that of its parent strain? Studies have shown that virulent *Brucella* strains inhibit BCV-lysosome fusion in cells after infection, whereas vaccine strains cannot effectively prevent BCV-lysosome fusion [17]. In this study, we showed that the deletion of the *Omp19* gene reduced the virulence of *Brucella* 2308, but that *Brucella* 2308 Δ*Omp19* significantly promoted BCV-lysosome fusion, although it reduced the viability of *Brucella* in cells. Therefore, a lipoprotein may contribute to the intracellular trafficking of *Brucella* in macrophages. Research has shown that *Brucella suis*, which lacks the LPS O side-chain, does not enter the intracellular circulation through the mediation of lipid rafts, and that the BCVs that enter the cell fuse with lysosomes and dissolve [18,19]. Therefore, the knockdown of the *Omp19* gene alters the intrinsic properties of the outer membrane of *Brucella* 2308, and possibly affects the O antigen on LPS, thus reducing its utilization of lipid rafts, and effectively reducing the early survival of *Brucella* in macrophages. However, how the *Brucella* 2308 Δ*Omp19* affects the fusion of BCVs and lysosomes in mouse macrophages requires further study.

Brucella escapes immune surveillance to survive in host cells, a process that depends on the secretion of various effector proteins or virulence factors that interfere with host cell apoptosis [20]. The smooth type of *Brucella* inhibits the apoptosis of host cells [21], mainly through the function of a TNF-*α*-dependent O-side-chain polysaccharide, which is not available in the rough type of *Brucella*. We have shown that the virulence and cytotoxicity of the *Brucella* 2308 Δ*Omp19* mutant strain are reduced compared with those of its *Brucella* 2308 parent, but it does not affect apoptosis (results not shown), and the apoptosis rate was the same as that induced by its parental strain. Therefore, although the *Omp19* virulence protein is a key protein in the intracellular survival of *Brucella*, it does not improve the intracellular viability of *Brucella* by regulating the apoptosis of the host cells. *Brucella* infection can reduce the body's immunity, and *Omp19* is an important protein in the mechanism of *Brucella* infection. Therefore, whether silencing this gene affects the host's immunity has been unclear. A previous study showed that the immune capacity of the host directly affects the replication and internalization of intracellular bacteria [22], and a strong immune response can inhibit the development of a persistent *Brucella* infection [23]. In this study, we found that *Brucella* 2308 Δ*Omp19* induced

higher levels of INF-γ in mice than *Brucella* 2308. IFN-γ, a key Th1-type immune cytokine, is required for the bactericidal activity of macrophages [24]. High levels of INF-γ induce the host immune system to kill and eliminate *Brucella in vivo* and *in vitro*, as shown in *Fig. 6*. Thus, the knockdown of the *Omp19* gene reduces *Brucella* 2308 virulence and activates the immune response of the host, this is a possible that B*rucella* 2308 Δ*Omp19* strain has potential utility as an attenuated vaccine against *Brucella*. The knockdown of gene *Omp19* in *Brucella* changes the intrinsic characteristics of the bacterial membrane, which affects its intracellular viability and induces the Th1 immune response. In further studies in livestock, we will investigate whether the *Brucella* 2308 Δ*Omp19* strain is a possible safe live candidate vaccine against *Brucella* infection.

In this paper, we describe the construction of the mutant *Brucella* 2308 Δ*Omp19* and the preliminary investigation of the mechanisms underlying its interaction with lysosomes. We evaluate the role of *Brucella* 2308 *Omp19* protein in intracellular survival, the host immune response in macrophages and mice. Our results provide information on the roles of the OMPs of *Brucella* in its pathogenic mechanism and may facilitate the development of a candidate vaccine against wild-type *Brucella*.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (U1803236), and the National Key Research and Development Plan (2017YFD0500304).

Conflict of Interest

The authors have declared that no competition interests.

Author Contributions

In the process of writing the article, Wang Yueli, Ma Zhongchen and Zhang Huan are responsible for the provision, integration and writing of the article data, Li Tiansen is responsible for the research conception and design, Chen Chuangfu is the article reviewer, and the other authors provided help in the trial process.

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