Characterization of novel Omp31 antigenic epitopes of *Brucella melitensis* by monoclonal antibodies

Jinfeng Li¹, Feihuan Hu¹, Shouyi Chen², Peifang Luo¹,³, Zuoping He¹, Wenjing Wang¹*, Jean-Pierre Allain¹,⁴ and Chengyao Li¹,⁵*

**Abstract**

**Background:** Brucellosis is a severe zoonotic disease worldwide. Detection and identification of *Brucella* species are essential to prevent or treat brucellosis in humans and animals. The outer membrane protein-31 (Omp31) is a major protein of *Brucellae* except for *B. abortus*, while the Omp31 antigenic epitopes have not been extensively characterized yet.

**Results:** A total of 22 monoclonal antibodies (mAbs) were produced against Omp31 of *Brucella* (*B. melitensis*), of which 13 recognized five linear epitopes, 7 reacted with semi-conformational epitopes and 2 reacted with conformational epitopes, respectively. The mAb isotypes were 11 (50%) IgG2a, 5 (23%) IgG1 and 6 (27%) IgM. On the basis of epitope recognition and reactivity levels, 8 mAbs including 3 IgM and 5 IgG clones were considered as highly reactive and potentially diagnostic antibodies. Among these mAbs, 7A3 (IgG1), 5B1 (IgG2a), 2C1 (IgG2a) and 5B3 (IgG2a) reacted with differently conserved linear epitopes of *B. melitensis*, *B. ovis*, *B. suis* and *B. canis* strains, while 5H3 (IgG2a) highly reacted with a conformational epitope of Omp31 when tested with several immunoassays.

**Conclusions:** These potent monoclonal antibodies can be used for identifying Omp31 antigens or detecting *B. melitensis* and other *Brucella* species beyond *B. abortus* in vitro or in vivo.

**Keywords:** *Brucella melitensis*, Omp31 antigen, Monoclonal antibody, Epitope identification, Conserved epitope

---

**Background**

Brucellosis is one of the most serious zoonoses worldwide. It causes severe diseases in humans and substantial animal losses. In China, human brucellosis cases are increasing. In 2015, 56,989 new cases of human brucellosis have been reported according to Chinese CDC (http://www.chinacdc.cn/), of them are mostly caused by *Brucella* (*B. melitensis*) infected sheep and goats or their products [1–3].

The 31–34 kDa outer membrane protein (OMP) or (Omp31) is a major membrane protein of *Brucellae* except for *B. abortus* [4]. Omp31 plays an important role in cellular and humoral immune protective responses against *Brucella* infection [4–11]. Previously we fully evaluated Omp31 epitopes in specific T-cell response in sheep vaccinated with attenuated *B. melitensis* vaccine [12]. However, the B-cell epitopes have not yet been extensively investigated. To date, only few epitopes recognized by antibodies to *Brucella* Omp31, such as monoclonal antibody A59/10F09/G10 recognizing amino acid 48–83 of *B. melitensis* M16 and presenting protective activity were reported [4, 13, 14]. In this study, we generated and characterized 22 novel murine monoclonal antibodies (mAbs) binding native Omp31 of *B. melitensis*. Some of these antibodies presented high reactivity with different epitopes of Omp31, suggesting potential capacity to identify Omp31 antigens of *Brucellae* or to detect *B. melitensis* or other *Brucella* species beyond *B. abortus*.

**Results**

**Production of mAbs to Omp31 of *B. melitensis***

A total of 22 mAbs reactive with recombinant Omp31 (rOmp31) were selected from screening of hybridomas.
by EIA (Table 1). Antibody isotypes were identified for all 22 mAbs, including 50% (11/22) IgG2a, 23% (5/22) IgG1 and 27% (6/22) IgM, respectively.

**Classification of Omp31 epitopes by mAb’s recognition**

In order to classify the Omp31 antigenic epitopes, all mAbs were tested for reactivity with 27 16mer overlapping peptides derived from full-length amino acid (aa) sequence, denatured or non-denatured protein forms of *B. melitensis* Omp31 in various immunoassays. Thirteen mAbs were reactive with 7 linear peptides in Peptide-ELISA (Fig. 1a). Twenty mAbs were reactive to the denatured rOmp31 and 14 mAbs to the denatured native membrane protein extract (NMP) by Western blot (Fig. 1b), respectively. The mAbs reactivity was also tested against the non-denatured native antigens in ELISA using the NMP or the supernatant of sonicated proteins (SSP) from *B. melitensis*, which showed that 16 and 20 mAbs were reactive, respectively (Fig. 1c).

According to the nature of Omp31 antigens recognized by 22 mAbs, the epitopes were stratified into three groups of linear (L), semi-conformational (SC) and conformational (C) forms. Among these 22 mAbs, 13 reacted with the linear epitopes, 7 reacted with the semi-conformational and 2 reacted with the conformational epitopes presented in either rOmp31 or native Omp31 antigens of *B. melitensis* (Table 1).

**Linear epitope mapping of Omp31 by mAbs**

Among seven reactive linear peptides (Fig. 1a), the epitope shared by peptides P05 and P06 was reactive with mAbs 1H2, 2D2, 2G9 and 7A3. However, due to the stronger reactivity with P05 than P06, the minimal aa common sequence of Omp31 was designated as epitope Ep5 (S^5^WTG^5^GYI^5^G^5^INA^5^60) (Fig. 2). Similarly, epitope Ep20 (G^1^6^8^GDASALHT^1^7^6^9) overlapped by peptides P19 and P20 reacted with mAbs 2C1, 2E7, 4E9, 4H10 and 8F11. Epitope Ep21 (AG^1^8^AG^1^GW^1^TL^1^G^1^G^1^AGE^1^2^0^2^9) reacted with both mAbs 2A8 and 6D8. Epitopes Ep11 (Q^8^AG^8^YN^8^WN^8^Q^8^LDN^8^G^8^V^8^VL^8^GA^8^0^2^0^2^) and Ep24 (E^2^0^4^E^2^LY^2^T^2^DL^2^G^2^K^2^RN^2^L^2^N^2^V^2^D^2^D^2^V^2^D^2^2^1^9^) were recognized only by mAb 5B1 or 5B3, respectively (Fig. 2). Alignment of Omp31 aa sequences showed that

**Table 1** Characterization of mAbs reactive to the epitopes within Omp31 of *B. melitensis*

| MAb    | Isotype | Peptide ELISA | rOMP31 Western-Blot | NMP Western-Blot | NMP ELISA | SSP ELISA | TC IFS | B. m ICS | Epitope (aa)/peptide       |
|--------|---------|---------------|---------------------|-----------------|-----------|----------|--------|---------|---------------------------|
| 1H2    | IgG1 (K)| ++           | –                   | –               | <1        | 1        | +      | –       | L (33–48/42–57)/P05/06    |
| 2D2    | IgM (K) | ++           | ++                  | ++              | 4.5       | 4.8      | +      | ++      | L (33–48/42–57)/P05/06    |
| 2G9    | IgG1 (K)| ++           | –                   | –               | <1        | 2.2      | +      | +       | L (33–48/42–57)/P05/06    |
| 7A3    | IgG1 (K)| ++           | ++                  | ++              | 7         | 17       | +      | +       | L (33–48/42–57)/P05/06    |
| 5B1    | IgG2a (K)| 3.8        | +                   | –               | 1.2       | 2        | +      | ++      | L (87–102)/P11            |
| 2C1    | IgG2a (K)| 2.7        | ++                  | ++              | 2.4       | 5.5      | ++     | ++      | L (159–174/168–183)/P19/20|
| 2E7    | IgG2a (K)| 1.8        | ++                  | ++              | 1.5       | 3.5      | ++     | ±       | L (159–174/168–183)/P19/20|
| 4E9    | IgG2a (K)| 3.7        | +                   | ++              | 2         | 5        | ++     | ++      | L (159–174/168–183)/P19/20|
| 4H10   | IgG2a (K)| 1.9        | ++                  | ++              | 2.2       | 4.2      | ++     | –       | L (159–174/168–183)/P19/20|
| 8F11   | IgG2a (K)| 2.2        | ++                  | ++              | 2.5       | 5.5      | ++     | +       | L (159–174/168–183)/P19/20|
| 2A8    | IgG1 (K)| 7.8        | ++                  | +               | <1        | 1.5      | (+)    | +       | L (177–192)/P21           |
| 6D8    | IgG1 (K)| 4.7        | ++                  | –               | <1        | <1       | +      | ±       | L (177–192)/P21           |
| 5B3    | IgG2a (K)| 2.8        | ++                  | +               | <1        | 16       | ++     | +       | L (204–219)/P24           |
| 2B6    | IgM (K) | <1          | ++                  | –               | 8         | 11       | –      | +       | SC(20–240)                |
| 4H6    | IgM (K) | <1          | ++                  | –               | <1        | 1.2      | –      | ±       | SC(20–240)                |
| 5B11   | IgM (K) | <1          | ++                  | –               | 5.8       | 9.2      | –      | –       | SC(20–240)                |
| 5H3    | IgG2a (K)| <1        | ++                  | +               | 11        | 18       | +      | +       | SC(20–240)                |
| 6E10   | IgG2a (K)| <1        | +                   | +               | 1         | 2        | ++     | –       | SC(20–240)                |
| 6F9    | IgG2a (K)| <1        | +                   | ++              | <1        | 1.5      | ++     | ±       | SC(20–240)                |
| 11C4   | IgG2a (K)| <1        | ++                  | ++              | 1.2       | 2.6      | ++     | –       | SC(20–240)                |
| 2H5    | IgM (K) | <1          | –                   | –               | 4.1       | 7        | –      | –       | C (20–240)                |
| 5F11   | IgM (K) | <1          | –                   | –               | 8.2       | 12       | –      | +       | C (20–240)                |

NMP, native membrane proteins extracts of *B. melitensis*; SSP, supernatant of sonicated *B. melitensis* proteins; TC IFS, Lentivirus-mediated Omp31, transduced 293T cells detected by immunofluorescent staining; Bm ICS, *B. melitensis* strain detected by immunochemo staining; L, linear; SC, semi-conformational; C, conformational. The numbers indicate the S/CO values in the EIA; the reactivity levels are indicated by ++ (strongly reactive), + (reactive), ± (indeterminate) or – (non-reactive) in the Western-blot, IFS and ICS. (+), indicate false positive for the negative control of cells. Five IgG and three IgM clones in bold are representatives of high capacity of mAbs reacting with native Omp31 antigens of *B. melitensis* in various assays.
these five epitopes were completely conserved among
*B. melitensis*, *B. ovis*, *B. suis* and *B. canis* except for a single aa
mutation (S172P) within Ep20 of *B. ovis* strains (Fig. 3).

**Recognition of Omp31-lentivirus transduced cells**

To detect *Brucella* Omp31 intracellularly, 293FT cells
were transduced by recombinant Omp31-lentivirus (LV-
HAGE-Omp31) for mimicking *Brucella* infection in hu-
man or animal cells. By using IFS, one IgM mAb (2D2)
and 16 IgG mAbs were reactive to the expressed
rOmp31 in transduced 293FT cells (Fig. 4).

**Identification of *Brucella melitensis* strains by mAbs**

To identify reactivity of mAbs with Omp31 on the mem-
brane of bacteria, the intact *B. melitensis* strains were
immunologically stained by ICS with mAbs, individually.
Of 22 mAbs, 12 were reactive with intact *B. melitensis*
bacteria by ICS (Fig. 5 and Table 1).

Based on cross-matching reactivity levels of mAbs to
the native Omp31 antigen carrying different recognition
epitopes, 1 IgG1 (mAb 7A3) and 4 IgG2a (mAbs 5B1,
2C1, 5B3 and 5H3) clones presented high reactive pro-
files suitable as diagnostic antibodies in immunoassays of
Western-blot, ELISA, IFS or ICS (Table 1). Among
six IgM clones, mAbs 2D2, 2B6 and 5F11 showed high-
level reactivity in EIAs (Table 1), making them more
suitable as primary antibodies for capturing the Omp31
antigen in serological tests. In contrast, mAbs 6D8, 2A8
and 4H6 were unable or weakly to react with the native
NMP, SSP or intact *B. melitensis* strains, suggesting that
the recognizing epitopes might not be exposed on the
protein surface of Omp31 or *Brucella* strains (Table 1).

**Discussion**

Since Omp31 was identified in *B. melitensis* [4], most
studies focused on its role in cellular immune protection
against *Brucella* infections [7, 8, 10, 12]. Although
Omp31 elicited specific antibody production and bac-
tericidal activity [6, 11, 15], it was considered a poor
diagnostic antigen [14]. In this study, we prepared 22

---

**Fig. 1** Reactivity of mAbs to Omp31 antigens of *B. melitensis*. (a) Binding of mAbs to 16mer overlapping peptides derived from Omp31 in Peptide-ELISA. (b) Identification of mAbs reacting with rOmp31 and NMP (native membrane protein extracts of *B. melitensis* M5–90) by Western-blot. (C) Reactivity of mAbs to NMP and SSP (supernatant of sonicated proteins of *B. melitensis* M5–90) by ELISA. NS3, an HCV NS3 peptide, recombinant protein and an un-related mAb to HCV NS3 were used as negative-controls, respectively. The dotted line indicates the level of cut off defined as mean + 2SD of OD value to negative controls.

**Fig. 2** Mapping for linear epitopes of Omp31 recognized by mAbs. The amino acid (aa) sequences of 16mer peptides reactive to the mAbs are presented, of which the epitopes (Ep) are designated on the top of underlined aa sequences. Aa position of Omp31 is indicated at the beginning and the end of the peptide sequence. MAbs are indicated below the epitopes they recognize.
murine mAbs against Omp31 of *B. melitensis*, of which 50% (11/22) were IgG2a, 23% (5/22) IgG1 and 27% (6/22) IgM isotypes, respectively. In contrast, we previously found IgG1 to be the majority of isotypes (62% or 18/29) of mAbs to BP26 of *B. melitensis* [16], which might in part explain why BP26 was a diagnostic antigen with higher reactivity to the sera from *Brucella* infected humans or animals. In subclasses of antibodies, mice have IgG1, IgG2a, IgG2b and IgG3, which are functionally similar to human IgG1, IgG2, IgG4 and IgG3, respectively. In general, IgG1 is mainly associated with Th2 but IgG2a with Th1 profiles [17–19]. Therefore, a higher population of IgG2a induced by Omp31 may confer Th1-type immune protection against *Brucella* infection through IFN-γ and up-regulating phagocytosis. However, BP26 mainly induced a major IgG1 subclass antibody and functionally polarized Th2 cells in *Brucella* infection [16–23]. The ratio of IgG isotypes might be varied in relating to different antigenic properties of proteins.

Besides cellular immune response, a specific monoclonal antibody to Omp31 (A59/10F09/G10, IgG2a) was identified previously as a protective factor against *B. melitensis* or *B. ovis* infection in mice [4, 14, 24]. MAb
A59/10F09/G10 recognized a hydrophilic loop minimized within aa 48–83 of Omp31, which was exposed on the surface of pathogenic strains of B. melitensis, B. ovis and B. canis [4, 13, 14, 25]. Another study reported that 11 mAbs obtained after immunization with truncated rOmp31 of B. ovis recognized epitopes localized at distant positions, such as aa48–83, aa149–182 or aa180–224 [14]. In our study, we depicted characteristics of 22 mAbs, and found that 13 mAbs were reactive with linear epitopes and others with conformational or semi-conformational epitopes [16]. None of the mAbs reacted with the same epitope as mAb A59/10F09/G10. However, we identified five new discreet epitopes (Ep) of Omp31 mapped within 27 16mer overlapping peptides, including Ep5 (aa39–49), Ep11 (aa87–102), Ep20 (aa168–176), Ep21 (aa183–192) and Ep24 (aa204–219) (Fig. 2). It remains to be investigated further whether some of mAbs reacting with these epitopes have the ability to protect animals against B. melitensis or B. ovis infection.

In addition, epitopes Ep5 and Ep21 were considered as B-cell epitopes in the present study and shared its aa sequences with Omp31 T-cell epitopes P06 and P21, which were identified in sheep vaccinated with attenuated B. melitensis M5–90 [12]. Interestingly, those mAbs recognizing both B and T cell epitopes were all of IgG1 isotype, while other mAbs to B-cell epitopes (Ep11, Ep20 and Ep24) were IgG2a isotype alone (Table 1). A 27 amino acid polypeptide (aa48–74) was previously identified as both T and B cell epitopes inducing cellular and humoral response in mice, and the IgG1 titer was higher than IgG2a in sera [26]. However, it is still unknown...
whether there are significant discrepancies between IgG1 and IgG2a binding to B or T cell epitopes, or iso-types of mAbs sharing reactivity with different types of epitopes. The mAb A59/10F09/G10 was IgG2a and reactive with a common epitope (aa48–83) of T and B cells [4], this finding being inconsistent with the above description of epitopes mostly associated with IgG1.

A recent study reported a monoclonal antibody-based *B. melitensis* lipopolysaccharide antigen detection by ELISA [27]. In our study, the panel of 22 mAbs to Omp31 was tested to evaluate their reactivity with different forms of recombinant and native Omp31 of *B. melitensis*, including intact bacteria and bacterial or cell extracts detected by Western-blot, ELISA, IFS and ICS. Data in Table 1 showed that 5 IgG and 3 IgM clones of mAbs had suitable ability for the detection of Omp31 or *Brucella* strains by diverse immunoassays. MAbs 7A3, 5B1, 2C1, 5B3 and 5H3 reacted with different Omp31 epitopes exposed on the surface of *B. melitensis* strain and identified by ICS. Few substitutions were found in alignments of Omp31 aa sequences of *B. melitensis*, *B. ovis*, *B. suis* and *B. canis* strains. The five new epitopes (Ep5, Ep11, Ep20, Ep21 and Ep24) identified in this study had conserved sequences except for a single aa mutation within Ep20 (Fig. 3), suggesting that these mAbs could be used to detect at least four species of *Brucella* strains. The IFS detection of intracellular *Brucella* might be an alternative assay of conventional bacterial culture for examination of *Brucella* elimination by brucellosis treatment in clinical practice [28].

**Conclusions**

This study identified 22 novel mAbs specific to Omp31 of *B. melitensis*. Five IgG and 3 IgM clones presented high ability to recognize multiple epitopes of Omp31 antigen, which were exposed on the protein surface of intact *B. melitensis* and highly conserved among *B. melitensis*, *B. ovis*, *B. suis* and *B. canis* strains. The monoclonal antibodies obtained in this study could provide a substantial help as key reagents in diagnostic tools for identifying *Brucella* Omp31 antigens in laboratory, or
for detecting intracellular Brucella melitensis and other Brucella species beyond B. abortus in clinical.

Methods

Animals
Mice were obtained from the Animal Experimental Center of Southern Medical University (SMU), Guangzhou, China. Animal care was in accordance with national and institutional policies for animal health and well-being. Mouse surgery was performed under anesthesia for minimizing suffering of animals.

Recombinant Omp31 (rOmp31)
The full-length gene of 221 amino acids (aa) encoding for the mature Omp31 (excluding 19 aa of signal peptides) from attenuated vaccine strain of B. melitensis M5–90 was constructed with pET-30a plasmid (pETOmp31) and expressed in E. coli as described previously [15, 16]. The soluble recombinant Omp31 was obtained at 95% purity and used for mouse immunization and serological tests.

Monoclonal antibody production
The 6-week old BALB/c female mice were immunized with three injections of rOmp31 antigen at 2-week intervals as previously described [15, 16]. The spleen cells prepared from the immunized mice were fused with SP2/0 myeloma cells by PEG 4000 (Sigma-Aldrich, St Louis, Missouri, United States). The hybridoma cells secreting monoclonal antibodies (mAbs) to rOmp31 were individually selected up to a single clone by EIA [15, 16]. All clones were passaged in producing cells for a period of 6 months and kept frozen in liquid nitrogen after which antibodies were kept frozen at −20 °C. Pre-immunization and immunized sera were collected and used as the negative or positive controls for screening mAbs, respectively. One mAb (IgG1 kappa) to recombinant non-structural protein-3 (NS3) of hepatitis C virus (HCV) was used as an unrelated negative control [29]. MAb isotyping was performed by IsoQuick Strips (Sigma-Aldrich, St Louis, Missouri, United States).

Lentivirus-mediated Omp31 expression ex vivo
Omp31 gene was transferred into a lentiviral vector and packaged as an infectious recombinant lentivirus LV-HAGE-Omp31. Omp31 was expressed in 293FT cells by Omp31-lentivirus-mediated transduction as described previously [30], mimicking Omp31 antigen in Brucella-infected mammalian cells.

Immunoaassays
Recombinant Omp31 and native Omp31-containing membrane protein extracts (NMP) from B. melitensis M5–90 were used in ELISA for mAbs detection [15]. A panel of 27 16mer overlapping peptides spanning full-length of Omp31 sequence were applied in peptide-ELISA to identify epitopes recognized by mAbs [12]. Cutoffs were calculated as mean OD + 2SD with 95% confidence interval (CI) of three negative controls. Western-blot was used to identify the reactivity of mAbs with rOmp31 or NMP extracted from transformed E. coli, B. melitensis or transduced cells [12, 30]. Lentivirus LV-HAGE-Omp31 transduced cells were detected by immunofluorescent staining (IFS) with individual mAbs. The intact B. melitensis strains were examined by immunochemo staining (ICS) under a microbiological optical microscope (Olympus, Japan).

A goat anti-mouse IgG and IgM horseradish peroxidase (HRP)-conjugate (Rockland Immunochemicals Corp, Boyertown, Pennsylvania, USA) was used as secondary antibody in ELISA and ICS. DyLightTM594-conjugated AffiniPure Goat Anti-Mouse IgG + IgM (H + L) (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) was used as secondary antibody in IFS. A mAb to HCV NS3 (IgG1) was used as negative control.

Abbreviations
B. Brucella spp; EIA: Enzyme immunoassay; ELISA: Enzyme linked immunosorbent assay; ICS: Immunochemo staining; IFS: Immunofluorescent staining; mAb: Monoclonal antibody; Omp31: Outer membrane protein 31

Acknowledgements
The authors thank for Drs Hao Zhang, Xiaoning Liu and Shuiping Hou (Guangzhou CDC, Guangzhou, China) for their preparing the slides of B. melitensis cultures for immunochemo staining in the study.

Funding
This study was funded by the grants from National Key Research and Development Program of China (No. 2017YFD050303), National Natural Science Foundation of China (No. 31372443), Guangdong Provincial S&T Development Program of China (No. 2017B030303003), Guangdong Provincial S&T Project (No. 2014A020214003), Guangzhou Key Laboratory for Blood Safety (No. 201509010009). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials
The monoclonal antibodies and hybridoma cells described in this study were available at the Department of Transfusion Medicine, Southern Medical University, Guangzhou, China.

Authors’ contributions
CL and WW conceived and designed the experiments. JL, FH, SC, PL and ZH performed the experiments. CL and WW analyzed the data. SC contributed reagents and materials. CL and JPA wrote the paper. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Mouse experimentation and sample collection were approved by Southern Medical University (SMU) Animal Care and Use Committee (permit numbers: NFYY-2008-043 and NFYY-2010-076).
Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

1Department of Transfusion Medicine, Southern Medical University, Guangzhou, China. 2Guangzhou Center of Disease Control and Prevention (CDC), Guangzhou, China. 3Qingsyuans People's Hospital, Qingsyuans, Guangdong, China. 4Department of Hematology, University of Cambridge, Cambridge, UK. 5School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, China.

Received: 8 July 2016 Accepted: 5 May 2017

Published online: 15 May 2017

References

1. Chen S, Zhang H, Liu X, Wang W, Hou S, Li T, Zhao S, Yang Z, Li C. Increasing threat of brucellosis to low-risk persons in urban settings. China. Emerg Infect Dis. 2014;20:126–30.
2. Deqiu S, Donglou X, Jiming Y. Epidemiology and control of brucellosis in China. Vet Microbiol. 2002;90:165–82.
3. Zhang WY, Guo WD, Sun SH, Jiang JF, Sun HL, Li SL, Liu W, Cao WC. Human brucellosis, Inner Mongolia, China. Emerg Infect Dis. 2010;16:2001–3.
4. Vizcaino N, Cloeckaert A, Zygmunt MS, Dubray G. Cloning, nucleotide sequence, and expression of the Brucella melitensis omp31 gene coding for an immunogenic major outer membrane protein. Infect Immun. 1996;64:3744–51.
5. Avila-Calderón ED, López-Merino A, Jain N, Peralta H, López-Villegas EO, Srrangananan N, Boyle SM, Witsontsky S, Contreras-Rodriguez A. Characterization of outer membrane vesicles from Brucella melitensis and protection induced in mice. Clin Dev Immunol. 2012;2012:352493.
6. Cassataro J, Pasquevich K, Bruno L, Wallach JC, Fossati CA, Baldi PC. Antibody reactivity to Omp31 from Brucella melitensis in human and animal infections by smooth and rough Brucellae. Clin Diag Lab Immunol. 2004;11:111–4.
7. Cassataro J, Estein SM, Pasquevich KA, Velkovksy CA, de la Barrera S, Bowden R, Fossati CA, Giambartolomei GH. Vaccination with the recombinant Brucella outer membrane protein 31 or a derived 27-amino-acid synthetic peptide elicits a CD4+ T helper 1 response that protects against Brucella melitensis infection. Infect Immun. 2005;73:8079–88.
8. Cassataro J, Velkovksy CA, de la Barrera S, Estein SM, Bruno L, Bowden R, Pasquevich KA, Fossati CA, Giambartolomei GH. A DNA vaccine coding for the Brucella outer membrane protein 31 confers protection against Brucella melitensis and B. abortus infection by eliciting a specific cytotoxic response. Infect Immun. 2005;73:6337–46.
9. Estein SM, Fiorentino MA, Paollicci FA, Clause M, Manazza J, Cassataro J, Giambartolomei GH, Coria LM, Zylberman V, Fossati CA, Kjekshus R, Goldberg FA. The polymorphic antigen BLSOmp31 confers protection against Brucella ovis infection in rams. Vaccine. 2009;27:6704–11.
10. Gupta VK, Rourt PK, Vihan VS. Induction of immune response in mice with a DNA vaccine encoding outer membrane protein (omp31) of Brucella melitensis. Indian J Med Res. 2005;79:305–13.
11. Tiwari S, Kumar A, Thavaseelam D, Mangali S, Rathod V, Prakash A, Barua A, Arora S, Sathyaseelan K. Development and comparative evaluation of a plate enzyme-linked immunosorbent assay based on recombinant outer membrane antigen Omp28 and Omp31 for diagnosis of human brucellosis. Clin Vaccine Immunol. 2015;22:1217–22.
12. Wang W, Wu J, Qiao J, Weng Y, Zhang H, Liao Q, Qiu J, Chen C, Albin JP, Li C. Evaluation of humoral and cellular immune responses to BP26 and OMP31 epitopes in the attenuated Brucella melitensis vaccinated sheep. Vaccine. 2014;32:825–33.
13. Cloeckaert A, de Wergifosse P, Dubray G, Limet JN. Identification of seven surface-exposed Brucella outer membrane proteins by use of monoclonal antibodies: immunogold labeling for electron microscopy and enzyme-linked immunosorbent assay. Infect Immun. 1990;58:3980–7.
14. Vizcaino N, Kistelberger R, Cloeckaert A, Marín CM, Fernández-Lago L. Minor nucleotide substitutions in the omp31 gene of Brucella ovis result in antigenic differences in the major outer membrane protein that it encodes compared to those of other Brucella species. Infect Immun. 2001;69:7020–8.
15. Estein SM, Cheves PC, Fiorentino MA, Cassataro J, Paolicchi FA, Bowden RA. Immunogenicity of recombinant Omp31 from Brucella melitensis in rams and serum bactericidal activity against B. abortus. Vet Microbiol. 2004;102:203–13.
16. Qiu J, Wang W, Wu J, Zhang H, Wang Y, Qiao J, Chen C, Gao GF, Alain JP, Li C. Characterization of periplasmic protein BP26 epitopes of Brucella melitensis reacting with murine monoclonal and sheep antibodies. PLoS One. 2012;7:e43246.
17. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature. 1996;383:787–93.
18. Banerjee K, Klasse PJ, Sanders RW, Pereyra F, Michael E, Lu M, Walker BD, Moore JP. IgG subclass profiles in infected HIV type 1 controllers and chronic progressors and in uninfected recipients of Env vaccines. AIDS Res Hum Retrov. 2010;26:445–58.
19. Viscaino ML, Tagliamonte M, Torresello ML, Buonaguro FM, Buonaguro L. Effects of adjuvants on IgG subclasses elicited by virus-like particles. J Transl Med. 2012;10:4.
20. Baldwin CL, Gokcen R. Host immune responses to the intracellular bacteria Brucella, does the bacteria instruct the host to facilitate chronic infection? Crit Rev Immunol. 2006;26:407–42.
21. Dunward MA, Harns M, Magnani DM, Eska L, Splitter GA. Discordant Brucella melitensis antigens yield cognate CD8+ T cells in vivo. Infect Immun. 2010;78:168–76.
22. Gupta VK, Radhakrishnan G, Harns J, Splitter G. Invasive Escherichia coli vaccines expressing Brucella melitensis outer membrane proteins 31 or 16 or periplasmic protein BP26 confer protection in mice challenged with B. melitensis. Vaccine. 2013;20:4017–22.
23. Pasquevich KA, Estein SM, García Samartino C, Zwerdling A, Coria LM, Barionuevo P, Fossati CA, Giambartolomei GH, Cassataro J. Immunization with recombinant Brucella species outer membrane protein Omp16 or Omp19 in adjuvant induces specific CD4+ and CD8+ T cells as well as systemic and oral protection against Brucella abortus infection. Infect Immun. 2009;77:436–5.
24. Bowden RA, Estein SM, Zygmunt MS, Dubray G, Cloeckaert A. Identification of protective outer membrane antigens of Brucella ovis by passive immunization of mice with monoclonal antibodies. Microbes Infect. 2000;2:481–8.
25. Bowden RA, Cloeckaert A, Zygmunt MS, Bernard S, Dubray G. Surface exposure of outer membrane protein and lipopolysaccharide epitopes in Brucella species studied by enzyme-linked immunosorbent assay and flow cytometry. Infect Immun. 1995;63:3945–52.
26. Cassataro J, Pasquevich KA, Estein SM, Laplagne DA, Velkovksy CA, de la Barrera S, Bowden R, Fossati CA, Giambartolomei GH, Goldberg FA. A recombinant subunit vaccine based on the insertion of 27 amino acids from Omp31 to the N-terminus of BL5 induced a similar degree of protection against B. ovis than rev.1 vaccination. Vaccine. 2007;25:4437–46.
27. Patra KP, Saito M, Atturi VL, Rolan HG, Young B, Kermines T, Smits H, Miccal JD, Gotuzzo E, Gilman RH, Tcolis RM, Vinetz JM. A protein-conjugate approach to develop a monoclonal antibody-based antigen detection test for the diagnosis of human brucellosis. PLoS Negl Trop Dis. 2014;8:e2926.
28. Castello MJ, Solera J. Chronic brucellosis and persistence of Brucella melitensis DNA. J Clin Microbiol. 2000;37:2084–7.
29. Bian Y, Zhao S, Zhu S, Zeng J, Li T, Fu Y, Wang Y, Zheng X, Zhang L, Wang W, Yang B, Zhou Y, Allain JP, Li C. Significance of monoclonal antibodies against the conserved epitopes within non-structural protein 3 helicase of hepatitis C virus. PLoS One. 2013;8:e70214.
30. Zhang L, Yin S, Tan W, Xiao D, Weng Y, Wang W, Li T, Shi J, Shuai L, Li H, Zhou J, Allain JP, Li C. Recombinant interferon-γ lentivirus co-infection inhibits adenoavirus replication ex vivo. PLoS One. 2012;7:e42455.