HOST RESPONSE TO INFECTION

Immunogenic properties of *Brucella melitensis* cell-wall fractions in BALB/c mice

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Summary. The immunogenicity of several *Brucella melitensis* cell-wall (CW) fractions was tested in BALB/c mice. These CW fractions were smooth lipopolysaccharide (S-LPS) fraction from smooth (S) *B. melitensis* strain 16M, sodium dodecyl sulphate-insoluble (SDS-I) CW fraction from *B. melitensis* strain 16M (S) undigested or digested with pepsin, and SDS-I CW fraction from rough (R) *B. melitensis* strain H38. The *B. melitensis* SDS-I CW fraction contained two major outer-membrane proteins (OMPs) of 25–27 kDa and 31–34 kDa, peptidoglycan (PG) and a small quantity (1.5%) of LPS. One month after immunisation, mice were challenged with virulent *B. melitensis* strain H38 (S) and *Brucella* spleen counts were recorded on days 28 and 49 after challenge. Before challenge, as measured by ELISA, the highest antibody responses to S-LPS were observed in mice immunised with SDS-I CW fraction from *B. melitensis* strain 16M (S), whether digested with pepsin or undigested. All immunised mice, except those immunised with the SDS-I CW fraction from the R strain, showed higher IgG1 than IgG2a antibody responses to S-LPS (IgG1:IgG2a ratio 3.64–7.71). Antibody responses to the 25–27-kDa OMP were very low, with the highest responses in the mice immunised with the SDS-I CW fraction from the R strain. These results indicated that, in BALB/c mice, these CW fractions probably induced Th2-dependent more than Th1-dependent antibody responses. The highest protection levels were produced by immunisation with the pepsin-digested SDS-I CW fraction from *B. melitensis* strain 16M (S) and by passive transfer of an anti-S-LPS monoclonal antibody (MAb 2E11; M epitope specificity). S-LPS fraction from *B. melitensis* strain 16M (S) or SDS-I CW fraction from *B. melitensis* strain H38 (R) did not induce significant protection. The absence of protection by S-LPS fraction could be explained by the low antibody titres induced by it. On day 49, protection conferred by pepsin-digested SDS-I CW fraction from *B. melitensis* strain 16M (S) was significantly higher ($p < 0.05$) than by the untreated SDS-I CW fraction from the corresponding strain. Furthermore, the level of protection in mice immunised with the SDS-I CW fraction from the R strain and which were also passively immunised, 1 day before challenge, with the S-LPS specific MAb was lower than in mice treated with the anti-S-LPS MAb alone. These results indicate that immunity induced by the S strain *B. melitensis* SDS-I CW fraction is probably mainly S-LPS dependent and that even one or more protein components of this fraction may play an antagonistic role to immunity conferred by S-LPS.

Introduction

Brucellae are gram-negative facultative intracellular bacteria which develop mainly in the reticulo-endothelial system. Dissemination to other target organs, such as joints and the genital tract, may also occur. Infection of the placenta may lead to abortion in pregnant animals of susceptible species (cattle, sheep and goats). *Brucella melitensis* is the major species

involved in caprine and ovine brucellosis and also causes severe infections in man.

The brucella cell wall (CW) consists of a peptidoglycan (PG) layer strongly associated with the outer membrane.¹ The outer membrane contains lipopolysaccharide (LPS), proteins and phospholipids. Outer-membrane proteins (OMPs) identified are the major proteins of 25–27, 31–34 and 36–38 kDa, which are also named group 3 and group 2 (porins) proteins, respectively,^{2–8} and minor proteins of 10, 16.5, 19 and 89 kDa.² All these OMPs have been shown to be

surface-exposed.² The sodium dodecyl sulphate-insoluble (SDS-I) CW fraction of *B. melitensis* is composed mainly of the two major OMPs of 25–27 and 31–34 kDa tightly bound to PG (30%), PG (50%) and a small quantity of LPS (< 2%).⁹ The major 36–38-kDa OMP (porin) of *B. abortus* is less abundant in *B. melitensis* strains.^{6,9} In addition, the 31–34 kDa OMP is a minor OMP in *B. abortus* strains but a major one in *B. melitensis* and the other *Brucella* spp.⁹

The SDS-I CW fraction of *B. abortus* has been reported to produce highly effective short- and long-term protection in mice.^{10–13} It was speculated that antigens involved in protection could be the major OMPs of this fraction. However, SDS-I CW fractions isolated from rough (R) *B. abortus* strains were shown to be much less protective than those isolated from smooth (S) strains which induced high titres of anti-S-LPS antibodies.^{9,14} By passive immunisation experiments, monoclonal antibodies (MAbs) to S-LPS epitopes have been shown to be highly protective in mice against both *B. abortus* and *B. melitensis* virulent S strains,^{14–19} whereas MAbs to OMPs and rough LPS (R-LPS) were ineffective or less protective.^{17,20–22}

The purpose of the present study was to investigate the immunogenicity of *B. melitensis* R and S strain CW fractions (SDS-I and S-LPS) in BALB/c mice. To determine the potential protective role of the major OMPs in the SDS-I CW fraction, long-term protection (28 and 49 days post immunisation) against a *B. melitensis* S virulent challenge was evaluated by active immunisation with untreated SDS-I CW fractions from S and R *B. melitensis* strains, with pepsin-digested SDS-I CW fraction from the S strain, with S-LPS from the S strain and by passive immunisation with an S-LPS specific MAb after active immunisation or not with the SDS-I CW fraction from the R strain.

Materials and methods

Bacterial strains

Smooth (S) and rough (R) strains—*B. melitensis* H38 (R) and (S), *B. melitensis* 16M (S), *B. melitensis* B115 (R)—were obtained from the strain collection maintained by J. M. Verger and M. Grayon at the Institut National de la Recherche Agronomique, Laboratoire de Pathologie Infectieuse et Immunologie, Nouzilly, France.

Cell-wall fractions

SDS-I CW fractions from *B. melitensis* H38 (R), *B. melitensis* B115 (R) and *B. melitensis* 16M (S) were extracted as described previously.^{5,11} Briefly, cells were killed by heating at 65°C for 1 h and broken with glass beads in a Braun MSK homogeniser or a Dyno-Mill apparatus (W. A. Bachofen, Basel, Switzerland). Crude CW were recovered by centrifugation (53 000 g, 4°C for 1.5 h). SDS-I CW fractions were obtained by treating crude CW in SDS 4% solution in the Laemmli

sample buffer for electrophoresis. Insoluble fractions were recovered by centrifugation, washed six times in distilled water, and then lyophilised. Pepsin-treated SDS-I CW fraction of *B. melitensis* 16M (S) was prepared by incubating for 10 min at room temperature 10 mg of the SDS-I CW fraction suspended in 1 ml of NaCl 0.9% with 2 ml of pepsin 10 mg/ml in 0.3 M HCl. After incubation, neutralisation with 1 ml of 1.85 M Tris and centrifugation, the pellet was washed five times in phosphate-buffered saline (PBS), pH 7.2. One part of these SDS-I CW fractions was treated further with lysozyme as described by Dubray and Charriaut⁵ for ELISA, SDS-PAGE and immunoblot analysis.

S-LPS fraction from *B. melitensis* 16M (S) was prepared by the phenol-water method.²³

Purification of the 25–27-kDa OMP

The 25–27-kDa OMP was purified from the *B. melitensis* B115 (R) SDS-I CW fraction as described previously.³

MAbs

Anti-OMP, anti-S-LPS and anti-R-LPS MAbs were produced as described previously.^{2,15,21} The S-LPS specific MAb 2E11 (IgG3) used in the passive protection experiments, shown to be highly specific for the M dominant S-LPS of *B. melitensis*,^{15,16} was produced as described by Limet *et al.*¹⁶ The S-LPS specific MAb 12G12 (IgG1) used in ELISA was specific to a common (C) epitope of A- and M-dominant S-LPS.¹⁵

ELISA

ELISA was performed as described previously.² The effect of pepsin treatment of the SDS-I CW fraction was determined by ELISA reactivity of a set of MAbs specific for the 25–27-kDa and 31–34-kDa OMPs, for S-LPS and for R-LPS on undigested and pepsin-digested SDS-I CW fraction of *B. melitensis* 16M (S) coated at a concentration of 20 µg/ml. For the antibody response study, S-LPS of *B. melitensis* 16M (S) or the purified 25–27-kDa OMP (both at a concentration of 1 µg/ml) were coated on microtitration plates (Greiner Labortechnik-Stuttgart) by overnight incubation at 37°C. After incubation for 2 h, binding of IgG1, IgG2a and IgG3 antibody of the mice sera (diluted 1 in 10, 1 in 100, 1 in 1000 and 1 in 10000) were revealed by further incubation for 1 h with biotinylated donkey anti-mouse IgG1, IgG2a and IgG3 immunoglobulins (Amersham) followed by incubation for 1 h with peroxidase-conjugated streptavidin (Amersham).

Latex agglutination immunoassay

The S-LPS content of the SDS-I CW fraction from *B. melitensis* 16M (S) was estimated by latex ag-

Fig. 1. Binding measured by ELISA of a set of MAbs (three-fold diluted hybridoma supernates) specific for the 25–27-kDa, 31–34-kDa and 36–38-kDa OMPs, S-LPS and R-LPS to undigested (■) and pepsin-digested (□) SDS-I CW fraction from *B. melitensis* 16M.

glutination inhibition assay as described previously²⁴ with S-LPS from *B. melitensis* 16M (S)-coated latex particles and MAb 2E11 as agglutinator. S-LPS from *B. melitensis* 16M (S) was used as a standard.

Immunoblot techniques

SDS-PAGE and immunoblotting were performed as described previously.²

Immuno-electronmicroscopy

Thin sections of undigested or pepsin-digested SDS-I CW fraction from *B. melitensis* 16M (S) were prepared and characterised by electronmicroscopy after immunogold labelling with MAbs specific for S-LPS (12G12) and for the 25–27-kDa OMP (A59/05F01/C09), as described previously.^{2,3}

Protection of mice

Groups of six BALB/c mice were actively immunised by subcutaneous injection of incomplete Freund's adjuvant with 50 µg of SDS-I CW fraction or 6 µg of S-LPS 1 month before standard intravenous challenge with *B. melitensis* strain H38 (S) (10⁴ cfu). Mice were bled at 1 day before and 49 days after

challenge to assess antibody responses against S-LPS and the 25–27-kDa OMP. Spleen counts were made 28 and 49 days after challenge. The experiment also included one negative control group that received saline in incomplete Freund's adjuvant, one group that was passively immunised with S-LPS specific MAb 2E11 (200 µl of ascitic fluid diluted 1 in 50 in saline) 1 day before the challenge and one group of mice immunised with the SDS-I CW fraction of *B. melitensis* strain H38 (R) that were also passively immunised with the S-LPS specific MAb. The statistical significance was determined after variance analysis (Dunnett's test).

Results

Effect of pepsin digestion of SDS-I CW fraction from *B. melitensis* 16M (S) on OMP, R-LPS and S-LPS content

Binding to undigested and pepsin-digested SDS-I CW fraction from *B. melitensis* 16M (S) of a set of MAbs directed to the 25–27-kDa, 31–34-kDa and 36–38-kDa OMPs, to R- and S-LPS was determined by ELISA. While showing variable binding (absorbance values of 0.25–2.50) to undigested SDS-I CW

Fig. 2. SDS-PAGE analysis with Coomassie Blue stain of undigested (lane 2) and pepsin-digested (3) SDS-I CW fraction from *B. melitensis* 16M; lane 1, M_r standards.

Fig. 3. Immunoblotting after SDS-PAGE of undigested (lanes 1, 3, 5 and 7) and pepsin-digested (2, 4, 6 and 8) SDS-I CW fractions from *B. melitensis* 16M with anti-25-27-kDa MAb A59/05F01/C09 (1 and 2), anti-31-34-kDa MAb A59/10F09/G10 (3 and 4), anti-36-38-kDa MAb A63/05A07/A08 (5 and 6), and anti-S-LPS MAb 2E11 (7 and 8).

fraction, seven of the 12 anti-25-27-kDa MAbs showed absence of reactivity to pepsin-digested SDS-I CW fraction (fig. 1). The other five MAbs retained very low antibody reactivity (absorbance value < 0.75) to the pepsin-digested SDS-I CW fraction. The anti-31-34-kDa MAb showed reduced antibody reactivity on the pepsin-digested SDS-I CW fraction but retained a higher antibody reactivity than the anti-25-27-kDa MAbs, whereas MAbs specific for R-LPS and S-LPS retained the same antibody reactivity as on undigested SDS-I CW fraction. MAbs specific for the 36-38-kDa OMP reacted weakly or not at all with both SDS-I CW fractions.

Lysozyme-digested SDS-I CW fraction (pepsin-digested or undigested) analysed by SDS-PAGE and Coomassie Blue staining or immunoblotting (figs. 2 and 3) with the MAbs confirmed the above results, i.e., the 25-27-kDa OMP bands were no longer visible after pepsin digestion whereas the 31-34-kDa OMP bands were diminished in their apparent molecular masses from 31-34 kDa to 22-30 kDa (figs. 2 and 3). The anti-36-38-kDa MAb did not react with undigested nor with pepsin-digested SDS-I CW fraction in immunoblotting (fig. 3). With the S-LPS-specific MAb, the same pattern of reactivity was observed whether digested with pepsin or undigested (fig. 3).

The S-LPS content in the SDS-I CW fraction was estimated at 1.5% by latex particle agglutination inhibition immunoassay with S-LPS of *B. melitensis* 16M (S) as a standard.

Ultrastructural examination of SDS-I CW fraction by immuno-electronmicroscopy

A thin-sectioned SDS-I CW fraction of *B. melitensis* strain 16M (S), digested with pepsin or undigested, was characterised by electronmicroscopy and immuno-gold labelling with MAbs specific for S-LPS (12G12) and for the 25-27-kDa OMP (A59/05F01/C09) (fig. 4). The structure resembled that of the *B. abortus* outer membrane-peptidoglycan complex described by Sowa *et al.*,⁷ i.e., a fibrillar structure consisting of single bilayers. The structure of the pepsin-digested SDS-I CW fraction was more loose than that of the undigested fraction. Both undigested and pepsin-digested SDS-I CW fractions were highly labelled by the S-LPS specific MAb 12G12. With MAb A59/05F01/C09, specific for the 25-27-kDa OMP, high labelling was observed only on the undigested SDS-I CW fraction, which is in accordance with the ELISA and immunoblotting results.

Protection induced by S-LPS and SDS-I CW fractions

On day 28 after challenge with virulent *B. melitensis* strain H38 (S), only BALB/c mice immunised with undigested or pepsin-digested SDS-I CW fractions from *B. melitensis* strain 16M (S) showed significantly reduced brucella spleen counts ($p < 0.05$) compared

Fig. 4. Immuno-electronmicroscopic examination of thin-sections of undigested (A and C) and pepsin-digested (B and D) SDS-I CW fraction from *B. melitensis* 16M; A and B, immunogold labelling with 25-27-kDa OMP-specific MAb A59/05F01/C09; C and D, immunogold labelling with S-LPS-specific MAb 12G12. Magnification $\times 73\,000$.

to unimmunised mice (table). Passive immunisation with anti-S-LPS MAb 2E11 (M epitope specific^{15,16}), 1 day before challenge, also resulted in significant protection ($p < 0.01$) whereas mice immunised with either S-LPS fraction or SDS-I CW fraction from *B. melitensis* strain H38 (R), or with the latter fraction

and passively immunised with MAb 2E11, were not significantly protected. Compared with unimmunised mice, spleen weights were significantly ($p < 0.01$ or $p < 0.05$) reduced in all groups of immunised mice except the mice immunised with SDS-I CW fraction from *B. melitensis* strain H38 (R) (table).

Table. Protective activity of *B. melitensis* cell wall antigens in mice against challenge with *B. melitensis* H38 (S)

Immunogen*	Mean (SD) log ₁₀ <i>Brucella</i> cfu/spleen		Mean (SD) spleen weight (mg)	
	28 days post-challenge	49 days post-challenge	28 days post-challenge	49 days post-challenge
Control	6.67 (0.26)	6.30 (0.39)	665 (158)	772 (230)
S-LPS 16M (S)	6.18 (0.47)	6.20 (0.62)	391 (56)§	675 (188)
SDS-I 16M (S)	5.75 (0.49)†	5.82 (0.31)	365 (206)§	586 (110)
Pepsin-digested SDS-I 16M (S)	5.80 (0.57)†	5.15 (0.65)§	387 (96)§	509 (112)†
SDS-I H38 (R)	6.77 (0.16)	6.89 (0.11)	752 (85)	1079 (148)§
SDS-I H38 (R) + anti-S-LPS MAb†	6.49 (0.31)	5.58 (0.34)†	422 (145)†	741 (169)
Anti-S-LPS MAb†	5.61 (0.91)§	5.19 (0.26)§	273 (99)§	739 (81)

*Mice were immunised with the CW fractions (single dose) 1 month before challenge.

†Anti-S-LPS MAb 2E11 (IgG3) was given 1 day before challenge.

‡p < 0.05 and §p < 0.01 in comparison with the untreated control group, as determined by Dunnett's test.

Fig. 5. IgG1 (▨), IgG2a (▩) and IgG3 (□) antibody reactivities (mean absorbance values and SD) to S-LPS of *B. melitensis* 16M measured by ELISA of sera from mice, either unimmunised (unimmunised-), immunised with S-LPS (S-LPS 16M (S)), undigested SDS-I CW fraction (SDS-I 16M (S)) or pepsin-digested SDS-I CW fraction (pepsin-SDS-I 16M (S)) from *B. melitensis* strain 16M (S) or SDS-I CW fraction from *B. melitensis* strain H38 (R) (SDS-I H38 (R)), taken 1 day before (1 in 100 diluted sera) (A) and 49 days after challenge (1 in 1000 diluted sera) (B) with *B. melitensis* strain H38 (S). The serum dilutions chosen were representative of antibody titre differences among the groups of mice.

Fig. 6. IgG1 (▨), IgG2a (▩), and IgG3 (□) antibody reactivities (mean absorbance values and SD) to the 25–27-kDa OMP of *B. melitensis* B115 measured by ELISA of sera from mice, either unimmunised (unimmunised-), immunised with S-LPS (S-LPS 16M (S)), undigested SDS-I CW fraction (SDS-I 16M (S)) or pepsin-digested SDS-I CW fraction (pepsin-SDS-I 16M (S)) from *B. melitensis* strain 16M (S) or SDS-I CW fraction from *B. melitensis* strain H38 (R) (SDS-I H38 (R)), taken 1 day before (1 in 10 diluted sera) (A) and 49 days after challenge (1 in 10 diluted sera) (B) with *B. melitensis* strain H38 (S).

On day 49 after challenge, mice showing significantly reduced spleen counts were those actively immunised with pepsin-digested SDS-I CW fraction from *B. melitensis* strain 16M (S) ($p < 0.01$) or with SDS-I CW fraction from *B. melitensis* strain H38 (R) and passively immunised with MAb 2E11 ($p < 0.05$) or passively immunised with MAb 2E11 alone ($p < 0.01$) (table). Only mice immunised with pepsin-digested SDS-I CW fraction from *B. melitensis* strain 16M (S) showed significantly ($p < 0.05$) reduced spleen weights compared with unimmunised control mice. However, mice immunised with SDS-I CW fraction from *B. melitensis* strain H38 (R) showed significantly increased ($p < 0.05$) spleen weights. On both days, 28 and 49 after challenge, immunisation with the latter fraction combined with passive immunisation with

MAb 2E11 resulted in a lower degree of protection than passive immunisation with MAb 2E11 alone.

Antibody responses to S-LPS and the 25–27-kDa OMP

The antibody responses to S-LPS measured by ELISA, 1 month after immunisation and 1 day before the challenge, were higher in BALB/c mice immunised with the *B. melitensis* strain 16M (S) SDS-I CW fraction, whether digested with pepsin or undigested, than in those immunised with the S-LPS fraction of the same strain (fig. 5A). The same antibody titres were observed with both undigested and pepsin-digested SDS-I CW fraction. The SDS-I CW fraction from the *B. melitensis* strain H38 (R) did not induce significant

antibody responses to S-LPS. The IgG1 antibody responses were always higher than the IgG2a antibody responses (IgG1:IgG2a ratio 3.64–7.71). The IgG3 antibody response level was between that of the IgG1 and IgG2a response level for the mice immunised with the SDS-I CW fraction and was the highest in mice immunised with the S-LPS.

The antibody responses to S-LPS increased *c.* 100-fold in all groups of mice 49 days after challenge (fig. 5B). The IgG1 antibody response remained higher than the IgG2a level in the mice immunised with *B. melitensis* strain 16M (S) SDS-I CW fractions but were equal both in the mice immunised with *B. melitensis* strain H38 (R) SDS-I CW fraction and in those immunised with S-LPS, whereas in the unimmunised mice the IgG2a anti-S-LPS antibody responses were higher than the IgG1 antibody responses (IgG1:IgG2a ratio of 0.51). In the three latter groups of mice, the IgG3 S-LPS antibody responses were the highest observed. In mice immunised with the *B. melitensis* strain 16M (S) SDS-I CW fraction, digested with pepsin or undigested, the IgG3 antibody response was equal to that of the IgG1 antibody response.

The antibody responses to the 25–27-kDa OMP were very low (titre giving an absorbance of $1.0 < 1$ in 10) in most groups of mice, both before and 49 days after the challenge (fig. 6). The highest antibody responses were observed 49 days after challenge in mice immunised with the SDS-I CW fraction of *B. melitensis* strain H38 (R).

Discussion

Protective activity of CW fractions of *B. abortus* against *B. abortus* infection in mice has been reported by several authors.^{9–11, 13, 25–28} S-LPS and, more precisely, the O-PS moiety has been shown to be an important protective antigen in the mouse model by passive immunisation with MAbs^{14–19, 22} or polyclonal antibodies¹² and by active immunisation with purified S-LPS^{9–11, 26–28} or O-PS conjugated to BSA.²⁹ Compared with *B. abortus*, the CW of *B. melitensis* has a different content of major OMPs and a different structure of the O-PS.³⁰ Whereas in *B. abortus* the major OMPs are the 25–27-kDa (group 3) and 36–38-kDa (group 2 porin) proteins, *B. melitensis* major OMPs are 25–27-kDa and 31–34-kDa proteins.⁹ These proteins have been found to be tightly bound to PG^{3, 7} and are present in the protective SDS-I CW fraction which consists of PG (50%) with the major OMPs (30%) linked to it and a small quantity of LPS (< 2%).⁹ In the present study we analysed the content of SDS-I CW fraction from *B. melitensis* by the use of MAbs. The poor representation of the 36–38-kDa major *B. abortus* OMP in the *B. melitensis* strain 16M (S) SDS-I CW fraction was confirmed. As judged by ELISA, SDS-PAGE and immunoblotting, the most abundant proteins were the major 25–27-kDa and 31–34-kDa OMPs. In addition, the SDS-I CW fraction

from *B. melitensis* strain 16M (S) contained both S-LPS and R-LPS molecules. Pepsin digestion affected the 25–27-kDa and 31–34-kDa major OMP content. However, the 31–34-kDa OMP seemed to be more resistant to pepsin digestion than the 25–27-kDa OMP. As examined by electronmicroscopy, the structure of the SDS-I CW fraction resembled that of the *B. abortus* outer membrane-peptidoglycan complex described by Sowa *et al.*,⁷ i.e., a fibrillar structure consisting of single bilayers. The structure of the pepsin-digested SDS-I CW fraction was more loose than that of the undigested fraction. This could be explained by loss of the tight interactions between major OMPs and PG. However, both undigested and pepsin-digested SDS-I CW fractions were strongly labelled by the S-LPS specific MAb 12G12. In addition, as shown by ELISA, pepsin digestion seemed not to affect the S-LPS or R-LPS content. Apparently S-LPS or R-LPS molecules interact to form stable complexes with PG. Interaction of LPS with the major brucella OMPs has been reported³¹ and loss of part of the major OMPs by pepsin digestion could, therefore, also possibly affect the LPS content of the SDS-I CW fraction. The nature of the interaction between LPS and pepsin-digested SDS-I CW fraction has yet to be determined.

The anti-S-LPS antibody responses were always higher in both undigested and pepsin-digested SDS-I CW fractions in sera from mice immunised with *B. melitensis* strain 16M (S) than in mice immunised with purified S-LPS. This is probably due to adjuvant activity of PG^{32, 33} present in the SDS-I CW fraction and not in the S-LPS fraction. The IgG1 antibody responses to S-LPS were also higher than the IgG2a antibody responses, which may be indicative of the predominance of immune responses of the Th2 type over the Th1 type.³⁴

The highest protection levels were obtained by immunisation with the pepsin-digested SDS-I CW fraction from *B. melitensis* strain 16M (S) and by passive transfer of an anti-S-LPS MAb (M epitope specific). Protective activity of the latter MAb in both BALB/c and CD-1 mice against a *B. melitensis* challenge was shown previously.^{15, 16} On day 49 after challenge, the protection conferred by pepsin-digested SDS-I CW fraction from *B. melitensis* strain 16M (S) was significantly higher ($p < 0.05$) than that given by the undigested SDS-I CW fraction of the corresponding strain. Furthermore, the level of protection in mice immunised with the SDS-I CW fraction from *B. melitensis* strain H38 (R) and which were also passively immunised, 1 day before challenge, with the S-LPS-specific MAb was lower than in mice treated with the anti-S-LPS MAb alone. The SDS-I CW fraction from *B. melitensis* strain H38 (R) did not induce significant protection. These results suggest that immunity induced by S strain *B. melitensis* SDS-I CW fraction is probably mainly S-LPS-dependent and that one or more components, probably protein, of this fraction may play an antagonistic role to immunity conferred

by S-LPS. Accordingly, the better protection conferred by S strain *B. melitensis* SDS-I CW fraction than by purified S-LPS fraction may be explained by the higher anti-S-LPS antibody titres induced by the former fraction. The antagonistic effect of a *B. abortus* surface protein called BCSP31 to immunity induced by S-LPS

has been reported previously.³⁵ It remains to be determined which proteins of the SDS-I CW fraction could play such a role.

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