INFECTION AND IMMUNITY, Jan. 1992, p. 312–315
0019-9567/92/010312-04$02.00/0
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Vol. 60, No. 1

Protection against *Brucella melitensis* or *Brucella abortus* in Mice with Immunoglobulin G (IgG), IgA, and IgM Monoclonal Antibodies Specific for a Common Epitope Shared by the *Brucella* A and M Smooth Lipopolysaccharides

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Received 20 May 1991/Accepted 7 October 1991

Mice passively immunized prior to a challenge infection with immunoglobulin G (IgG) and IgM monoclonal antibodies (MAbs) specific for a common epitope of both A- and M-dominant strains had viable *Brucella abortus* 544 or *Brucella melitensis* H38 counts in the spleen reduced to the same extent as did mice passively immunized with MAbs specific for either the A or the M epitope. The IgA MAb was not effective.

The smooth lipopolysaccharide (S-LPS) of smooth *Brucella* spp. has been found to contain two distinct epitopes designated A and M (2, 3, 5, 6, 9, 12). The relative amounts of the two epitopes vary among smooth *Brucella* strains, and these epitopes are absent on rough *Brucella* strains (1).

Monoclonal antibodies (MAbs) to S-LPS were previously shown to confer protection in mice by reducing the number of *Brucella* cells in the spleen and liver (7, 8, 10, 11). By using an anti-A, anti-M MAb, Limet et al. (7, 8) have demonstrated the importance of MAb specificity in conferring protection against challenge strains expressing mostly one or the other S-LPS epitope. We obtained MAbs of different isotypes and directed against a common epitope shared by A and M S-LPSs by fusion with NSO myeloma cells of spleen cells from mice infected with rough *Brucella melitensis* B115 (4). Our purpose was to evaluate in BALB/c mice the protective activity of these MAbs, characterized by immunoblotting and enzyme-linked immunosorbent assay (ELISA), against a *B. melitensis* or *Brucella abortus* virulent challenge strain expressing, respectively, the M or A epitope as the dominant S-LPS epitope and to compare their activity with that of MAbs specific for either the A or the M epitope (7).

In immunoblotting with two S-LPSs, i.e., S-LPS A of *B. abortus* 99 and S-LPS M of *B. melitensis* 16M, immunoglobulin G2a (IgG2a) MAb 04F9 (MAb-A) revealed only S-LPS A, IgG3 MAb 2E11 (MAb-M) revealed only S-LPS M, and IgG1 MAb 12G12 revealed both S-LPSs equally well (Fig. 1). IgA MAb 18H08 and IgM MAb 13G11 also revealed both S-LPSs (data not shown). In ELISA the three MAbs specific for the common epitope bound equally well to S-LPS from reference strains of *B. abortus* (99), *B. melitensis* (16M), and *Brucella suis* (biovar 4) (data not shown). The S-LPS structure has been defined as homopolymers of 4,6-dideoxy-4-formamido-α-D-mannopyranose residues. S-LPS A is a linear, α-1,2-linked polymer with about 2% α-1,3-linkages, while S-LPS M is a linear polymer of pentasaccharide repeating units containing one α-1,3-linked and four α-1,2-linked monosaccharide residues (2, 3, 9). Apparently, the α-1,3 linkage is the major part of the structure recognized by MAb-M. The percentage of α-1,3 linkages correlates with the reactivity of MAb-M with S-LPSs of *B. abortus*, *B. melitensis*, and *B. suis* expressing the M epitope in variable amounts (9; unpublished results). However, the structure recognized by MAbs specific for the epitope A and the common epitope has yet to be determined. Such a determination should be possible with synthetic oligosaccharides as described by Bundle et al. (2).

In ELISA MAb-M bound to *B. melitensis* H38 (M-dominant) cells at a high dilution but not to *B. abortus* 544 (A-dominant) cells (Fig. 2). One or two α-1,3 linkages occur per LPS chain in A-dominant strains (2). The very low binding of MAb-M to the A-dominant strain (10^5- to 10^6-fold lower than that to the M-dominant strain) could be explained by the fact that all α-1,3 linkages are not accessible to MAb-M on S-LPS A at the cell surface. MAb-A bound to both strains but had a 100-fold lower titer against *B. melitensis* H38. S-LPS MAbs 12G12, 13G11, and 18H08 bound equally well to both strains.

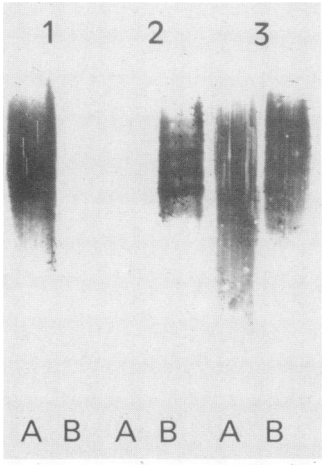

FIG. 1. Immunoblot of proteinase K-treated S-LPSs (6.25 μg) of *B. abortus* 99 (A) and *B. melitensis* 16M (B) with anti-S-LPS MAbs 04F9 (IgG2a, A epitope) (lane 1), 2E11 (IgG3, M epitope) (lane 2), and 12G12 (IgG1, common epitope) (lane 3).

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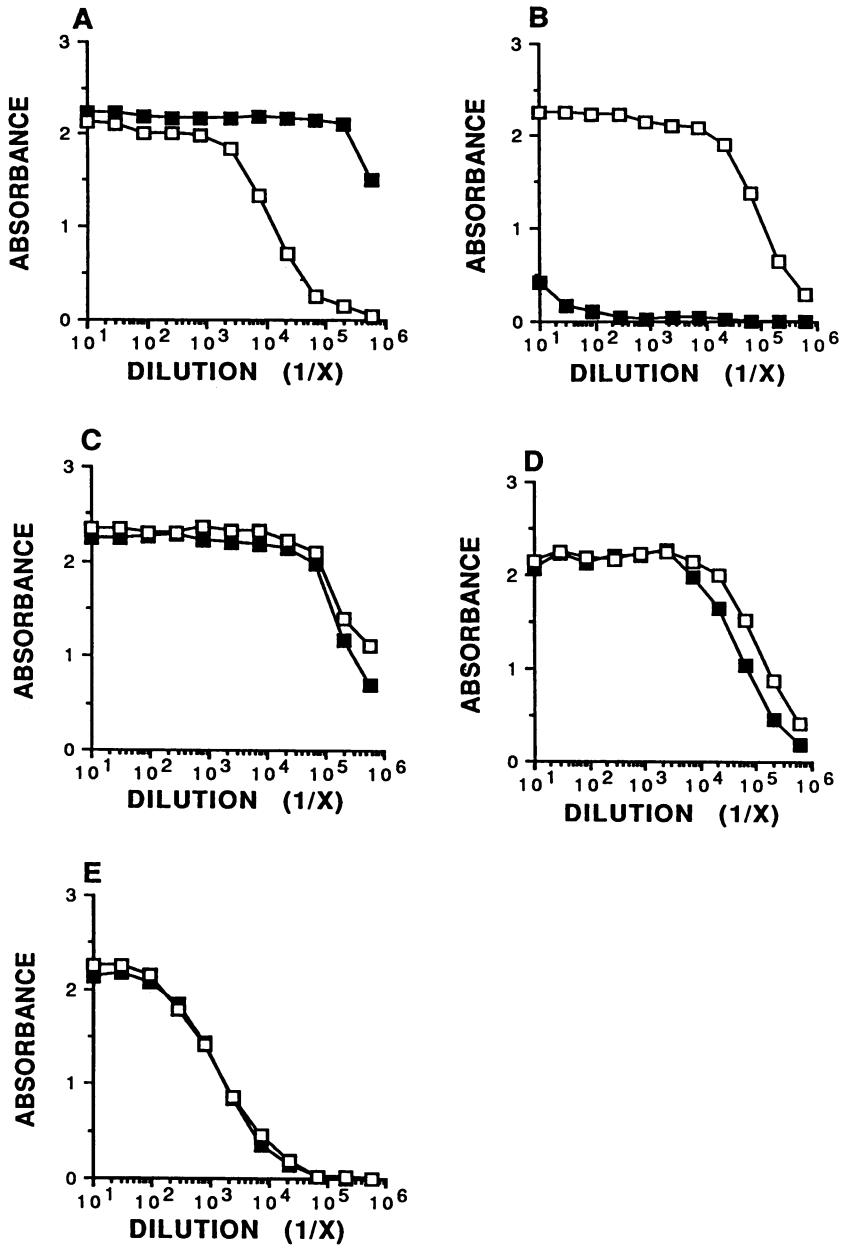

FIG. 2. Binding of the 04F9 (IgG2a, A epitope) (A), 2E11 (IgG3, M epitope) (B), 12G12 (IgG1, common epitope) (C), 13G11 (IgM, common epitope) (D), and 18H08 (IgA, common epitope) (E) anti-S-LPS MAbs (dilutions of ascitic fluids) to cells of *B. abortus* 544 (■) and *B. melitensis* H38 (□).

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TABLE 1. Protection conferred by anti-S-LPS MAbs against *B. melitensis* H38 (M dominant)

MAb[a]	Isotype	Specificity	Mean ± SD $\log_{10}$ *Brucella* CFU in the spleen at the indicated day postchallenge:		
			7	21	49
Control			5.70 ± 0.20	6.65 ± 0.11	6.35 ± 0.12
04F9 (MAb-A)	IgG2a	A	4.02 ± 0.06[b]	4.05 ± 0.22[b]	3.97 ± 0.59[b]
2E11 (MAb-M)	IgG3	M	4.32 ± 0.05[b]	4.70 ± 0.46[b]	5.42 ± 0.48[b]
12G12	IgG1	Common	4.15 ± 0.10[b]	3.69 ± 0.10[b]	5.16 ± 0.61[b]
18H08	IgA	Common	6.24 ± 0.10	6.76 ± 0.05	6.53 ± 0.29
13G11	IgM	Common	4.13 ± 0.03[b]	3.81 ± 0.27[b]	4.84 ± 0.53[b]

[a] MAbs (ascitic fluids) were injected 1 day prior to the *Brucella* challenge.
[b] $P < 0.01$ in comparison with the untreated control group, as determined by the F test.

In accordance with the reduction in the number of *Brucella* cells in the spleen, immunity conferred by MAb-A was highly significant ($P < 0.01$) against challenges of 2.45×10^4 CFU of *B. abortus* 544 and of 0.65×10^4 CFU of *B. melitensis* H38 at 7, 21, and 49 days postchallenge (Tables 1 and 2). Immunity conferred at 7, 21, and 49 days postchallenge by MAb-M was highly significant ($P < 0.01$) against M-dominant *B. melitensis* H38 but not against A-dominant *B. abortus* 544. IgG1 MAb 12G12 specific for the common epitope conferred highly significant protection ($P < 0.01$) against both challenge strains at 7, 21, and 49 days postchallenge; IgM MAb 13G11 did so against *B. melitensis* H38 at 7, 21, and 49 days postchallenge but against *B. abortus* 544 only at 7 days postchallenge; and the IgA MAb did not induce significant protection against either challenge strain. Although binding of IgM MAb 13G11 to both strains was approximately equal, better protection was observed against *B. melitensis* H38 than against *B. abortus* 544. This fact could be related to the virulence of these strains in BALB/c mice. When spleen counts were compared in control mice, *B. abortus* 544 seemed to be more virulent than *B. melitensis* H38. The MAbs most protective against the two challenge strains were MAb-A and IgG1 MAb 12G12 specific for the common epitope.

In a previous study with the same MAb-A and MAb-M, MAb-A conferred significant protection against *B. melitensis* H38 only at day 7 postchallenge (7). The injected dose of MAb-A was, however, very weak (4 μg), and the titer against *B. melitensis* H38 was very low (1/30). In contrast, the ascitic fluid that we used had a titer of 1/196,830 against this strain. Thus, when injected at high concentrations, MAbs specific for the A epitope can provide long-term protection in mice against a *B. melitensis* challenge when the A epitope, although not dominant, is accessible to antibodies at the bacterial cell surface. The same is not true for protection conferred by MAb-M. The absence of protection against *B. abortus* 544, which expresses the M epitope as a minor antigen, is probably related to the poor binding of MAb-M to whole cells of this strain. It was previously shown that MAb-M was protective against *B. abortus* 544 at 7 days postchallenge (7). The mice used for that experiment were, however, CD-1 mice, which are less sensitive to *B. abortus* 544 infection than the BALB/c mice used in this study, as indicated by the fact that a decrease (>1 log) in the number of *Brucella* cells in the spleen was already observed in control CD-1 mice at 21 days postchallenge. Therefore, to evaluate long-term protection against *B. abortus* 544, mice such as BALB/c mice seem to be more appropriate, since chronic infection can be established more easily.

The binding of MAbs to challenge strains seems to be requisite for good protection, but the degree of protection conferred is not directly related to the antibody titer against the challenge strains. Indeed, although better binding (higher titer) of MAb-M than of MAb-A was observed with *B. melitensis* H38 cells, MAb-A conferred better protection than did MAb-M against this strain at 7, 21, and 49 days postchallenge. This result could be explained by the fact that protection depends on the ability of the antibody to improve bacterial clearance. This phenomenon depends on both the ability of the antibody to bind bacteria and the ability of macrophage receptors to capture the opsonized bacteria. The functional affinity of the antibody and the abundance and accessibility of the A epitope on the challenge strain influence the first step, and the antibody subclass influences the second step. α-1,2-Linkages are probably involved in the structure of the A epitope, and the number of α-1,2-linkages always exceeds the number of α-1,3-linkages in the M-dominant strain. However, in the present case, the accessibility of the A epitope to the antibody is a prerequisite but is probably not the limiting factor, and it is worth noting that

TABLE 2. Protection conferred by anti-S-LPS MAbs against *B. abortus* 544 (A dominant)

MAb[a]	Isotype	Specificity	Mean ± SD $\log_{10}$ *Brucella* CFU in the spleen at the indicated day postchallenge:		
			7	21	49
Control			5.91 ± 0.18	7.63 ± 0.08	6.49 ± 0.09
04F9 (MAb-A)	IgG2a	A	4.70 ± 0.11[b]	4.67 ± 0.29[b]	4.97 ± 0.75[b]
2E11 (MAb-M)	IgG3	M	6.33 ± 0.11	7.89 ± 0.08	6.69 ± 0.13
12G12	IgG1	Common	4.75 ± 0.09[b]	5.20 ± 0.70[b]	5.66 ± 0.64[b]
18H08	IgA	Common	5.77 ± 0.16	7.50 ± 0.33	6.48 ± 0.16
13G11	IgM	Common	5.14 ± 0.19[b]	7.34 ± 0.11	6.56 ± 0.13

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *b*.

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the functional affinity rather than the intrinsic affinity or avidity is the most important factor affecting binding in vivo and subsequent protection.

Our results suggest that mainly IgG but also IgM antibodies against the common epitope of *Brucella* S-LPSs are efficient against the two *Brucella* species tested, which express the A and M epitopes in variable amounts. Therefore, vaccine development could be based on the common epitope and not only on either the A or the M epitope as suggested previously (7).

We are most grateful to J. M. Verger and M. Grayon for providing bacterial cultures and controls. We thank G. Bézard, A. Van Rompaye, and J. Van Broeck for expert technical assistance.

A. Cloeckaert was supported by the Institut National pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture.

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