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Borrelia burgdorferi Shows Specificity of Binding to Glycosphingolipids

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Live but not fixed or heat-killed *Borrelia burgdorferi* bound to galactocerebroside, lactosylceramide, and ceramide trihexoside. In addition, this organism bound to the disialoganglioside GD1a and the trisialoganglioside GT1b but not to gangliosides GM1, GD1b, GM2, and GM3 and not to asialo GM1. This adhesion pattern confirmed earlier findings of binding to galactocerebroside and places this organism within a prokaryotic group which binds to lactosylceramide. The binding to GD1a and GT1b, both of which carry terminal as well as multiple sialic acids, indicates that *B. burgdorferi* can show specificity of binding within a group of acidic gangliosides. Adhesion could not be inhibited by several concentrations of sugars and sialic acid, indicating more complex binding requirements than for terminal carbohydrates alone. Low-passage strains adhered to the four substrates in greater numbers than strains in culture for long periods of time. OspB mutants in general bound better or at least equally well to several of the glycosphingolipids, and preincubation of substrates with soluble recombinant and affinity-purified Osp did not inhibit or weakly inhibited the binding of the organisms. These findings suggest that outer surface lipoproteins A and B are not directly involved in adhesion to glycosphingolipids.

Borrelia burgdorferi, the causative agent of Lyme disease (2, 10, 40), binds to numerous types of cells (3, 17, 21, 23, 33, 44, 45) and substrates (11, 24, 28). This lack of adhesion specificity is consistent with the ability of this spirochete to invade and reside in the tissues of different organs. One approach to the understanding of this lack of cell adhesion specificity would be that *B. burgdorferi* uses receptors which may be common to most cells.

Glycosphingolipids are abundant on most types of cells, although some tissues may have greater or lesser concentrations of each, and some may be specifically associated with only one type of tissue (43). As receptors for bacteria, some glycosphingolipids can provide for specific interactions. Uropathogenic *Escherichia coli* can recognize prominent glycosphingolipids in the urinary tract epithelium (7), and lung-colonizing bacteria demonstrate high affinity for glycosphingolipids of the respiratory system (32). These specific adhesion patterns may be evidence of tissue tropisms.

One *Borrelia* (both *B. burgdorferi* and *B. hermsii*)-cell interaction appears to utilize galactocerebroside (GalCer) as a receptor (24). This molecule is but one of many surface glycosphingolipids which can act as receptors for different types of bacteria, bacterial toxins, and fungi (19, 27, 29, 30, 37, 41, 42).

Adhesion of *B. burgdorferi* to ubiquitous proteoglycans has been demonstrated (28), and more specifically, the binding of this organism to platelet integrins has recently been characterized (11). Binding of *B. burgdorferi* to glycosaminoglycans (28) and to glycosphingolipids (24) may represent very similar adhesion pathways since both types of molecules may be present in large numbers of cells.

While *B. burgdorferi* has been found to adhere to numerous and diverse types of cells, a borrelial ligand has not been identified with certainty for any of the cells or substrates studied so far, with the exception of a binding domain for endothelium in outer surface protein A (OspA) of the organism (16). The function(s) of the abundant OspA and OspB in *B. burgdorferi* are unknown, but their surface exposure makes them likely candidates for involvement in specific adhesion pathways. In addition, these two lipoproteins are basic and hence positively charged, a factor which could potentially contribute to their involvement in nonspecific ionic interactions (4, 6, 8). In this study, we have attempted to demonstrate the binding capacity of *B. burgdorferi* for glycosphingolipids and the potential role of OspA and OspB in binding to these molecules.

MATERIALS AND METHODS

Spirochetes. Strains used included B31 (10), a tick isolate from Shelter Island, N.Y., ESP1 (22), a tick isolate from Spain, and TI1 (13), a tick isolate from Montauk, N.Y. All are *B. burgdorferi* sensu stricto. In addition, mutants of each strain were created by monoclonal antibody (MAb) selection for OspB mutations according to the method of Coleman et al. (14). These mutant strains have been named EVB− (15), ESP1-EV, and TI1-EV, respectively. Both EVB− and ESP1-EV have point mutations in the *ospB* operon, resulting in premature stop codons and truncations in the OspB protein. TI1-EV has a deletion mutation in the *ospA-ospB* operon, resulting in a chimeric protein of approximately 34 kDa, similar to one described by Rosa et al. (39). PCR methods and primers used to characterize the mutant strains were as described by Coleman et al. (15).

EVB−, ESP1-EV, and TI1-EV have all retained their respective *ospB* mutations for several passages. All spirochetes were grown at 33°C in a serum-free medium that has been described previously (5).

Spirochetes were harvested from the medium by centrifugation at 7,000 × *g* for 20 min (25°C) and washed two times in phosphate-buffered saline (PBS) containing 5 mM magnesium chloride (pH 7.4). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done with a Laemmli buffer system (34) in gels of 10% acrylamide. All other conditions have been described previously (4, 12, 13). Reactivities of MAbs were determined by Western blot (immunoblot), also as described previously (3, 12, 13).

Affinity and recombinant OspA and OspB purification. Strain B31 was sonicated in a solubilization buffer consisting of 10 mM Tris HCl, 5 mM EDTA, 0.1% SDS, 0.2% deoxycholic acid, 2% Triton X-100, and 150 mM NaCl (all from Sigma, St. Louis, Mo.) and clarified by centrifugation at 10,000 × *g*. The antigen was then preadsorbed with 50 μl of a 10% solution of protein G-Sepharose (Pharmacia/LKB Biotechnology, Piscataway, N.J.) at 4°C. For affinity purification of OspA, an immunoglobulin M (IgM) MAb to OspA, 11G1, was purified from ascitic fluid by saturated ammonium sulfate precipitation and dialysis (4). Two milliliters of protein G-Sepharose was washed twice with distilled water and

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TABLE 1. Glycosphingolipids used as adherence substrates

Glycosphingolipid	Structure
Galactosylceramide (galactocerebroside)	Galβ1-1Cer
Lactosylceramide	Galβ1-4Glcβ1-1Cer
Ceramide trihexoside (globotriaosylceramide)	Galα1-4Galβ1-4Glcβ1-1Cer
Globoside	GalNacβ1-3Galα1-4Galβ1-4Glcβ1-1Cer
Monosialoganglioside GM1	Galβ1-3GalNacβ1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Cer
Monosialoganglioside GM2	GalNacβ1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Cer
Monosialoganglioside GM3	NeuAcα2-3Galβ1-4Glcβ1-1Cer
Asialoganglioside GM1	Galβ1-3GalNacβ1-4Galβ1-4Glcβ1-1Cer
Disialoganglioside GD1a	NeuAcα2-3Galβ1-3GalNacβ1-4[NeuAcα2-3]Galβ1-4Glcβ1-1Cer
Disalioganglioside GD1b	Galβ1-3GalNacβ1-4[NeuAcα2-8NeuAcα2-3]Galβ1-4Glcβ1-1Cer
Trisialoganglioside GT1b	NeuAcα2-3Galβ1-3GalNacβ1-4[NeuAcα2-8NeuAcα2-3]Galβ1-4Glcβ1-1Cer

once with 20 mM sodium phosphate buffer, pH 7.0, and incubated at room temperature with goat anti-mouse IgM (10 mg) (Cappel Laboratories, Durham, N.C.). Following two washes with phosphate buffer, the protein G-Sepharose was incubated and bound with 10 mg of purified 11G1. The protein G-Sepharose was then washed three times with phosphate buffers of increasing pH (7.5, 7.9, and 8.2) and resuspended in 5 ml of 20 mM dimethylpimelimidate HCl (Sigma), pH 8.6. Incubation was for 45 min at 4°C with gentle agitation. The protein G-Sepharose was then washed with 20 mM ethanolamine (Sigma) and twice with phosphate buffer, pH 7.0. The previously prepared borrelial antigen was then adjusted to 500 mM NaCl and incubated with the antibody-Sepharose matrix. After several washings with phosphate buffer, the mixture was poured into a column and OspA was eluted with 0.5-ml portions of 0.1 M glycine-HCl, pH 2.5. The fractions were restored to neutral pH by addition of 1 M Tris-HCl, pH 9.5. Purity was monitored by SDS-PAGE of the eluate with subsequent passes through the column until homogeneity was achieved. Affinity-purified products were stored at −70°C.

Identical procedures were used for the purification of OspB; however, an IgG MAb to OspB, CB2 (14), was used instead of 11G1 and bound directly to the protein G-Sepharose.

To ascertain that the affinity-purified Osp contained the posttranslation lipid modifications, the spirochetes were biosynthetically radiolabeled with [9, 10^{-3}H(*N*)]palmitic acid (Dupont-New England Nuclear, Boston, Mass.) and subjected to the same affinity purification procedures as those described above (13). The eluate was then electrophoresed and fluorographed.

Recombinant OspA and OspB, truncated at the NH_2 termini and thus unlipidated, were gifts from John J. Dunn (Brookhaven National Laboratories, Upton, N.Y.) (18).

Adhesion to glycosphingolipids. Flat-bottom 96-well polyvinyl chloride enzyme-linked immunosorbent assay (ELISA) plates (Falcon; Becton Dickinson, Oxnard, Calif.) were coated with glycosphingolipids (Table 1; all purchased from Matreya Inc., Pleasantville, Pa., and/or Sigma) at final concentrations of 0.5, 1.0, 1.5, and 2.0 μg per well in 100 μl of chloroform-methanol (1:1). The solvent was allowed to evaporate overnight at 4°C. Wells were blocked overnight at 4°C with 200 μl of PBS supplemented with 2% bovine serum albumin (BSA) and washed three times with PBS containing 0.05% Tween 20 prior to addition of spirochetes. A murine MAb to galactocerebroside (GalCer) (Boehringer Mannheim, Indianapolis, Ind.), rabbit polyclonal antisera to GM1 (Matreya, Inc.), and human serum with broad antiganglioside reactivity (25) were used to monitor the efficiency of glycosphingolipid deposition to the plates. Spirochetes, at a concentration of 3×10^6 in 50 μl of medium, were added to each well and incubated for 4 h at 33°C. Wells were washed two times with 200 μl of PBS-BSA, fixed with 10% buffered formalin for 30 min at room temperature, and washed three times with PBS-BSA. Adherence of spirochetes was measured by ELISA. A 100-μl portion of an IgG3 murine MAb directed against the flagellar antigen of *B. burgdorferi* (12) was added, plates were covered, incubated for 2 h in a humid chamber at 37°C, and washed three times with PBS-BSA, and 100 μl of peroxidase-conjugated, affinity-purified goat antibodies to mouse IgG (Kirkegaard and Perry, Gaithersburg, Md.) in PBS-BSA was added and incubated for 1 h. This was followed by the addition of 100 μl of substrate solution containing 0.04% *o*-phenylenediamine, 0.1 M sodium citrate, and 0.02 M Na_2HPO_4, pH 5, with 0.003% H_2O_2, incubation for 30 min at room temperature in the dark, and then addition of 30 μl of H_2SO_4. Optical density was measured spectrophotometrically at 492 nm in an MR 700 Microtiter plate reader (Dynatech Laboratories, Alexandria, Va.).

Each plate had chloroform-methanol-treated wells as blanks, BSA-coated wells, and wells to which no MAb was added but which received both conjugates and substrates as controls. Spirochetes were heat killed (incubated at 56°C for 30 min) or fixed in 1% buffered formalin for assays requiring nonviable organisms.

For analysis, the mean optical density obtained for control BSA wells was subtracted from the mean optical density obtained for each of the glycosphingolipids. The optical densities expressed in the figures are actually the differences between the mean optical density of the glycosphingolipid and the mean values obtained for the BSA controls. Thus, in some instances, negative values for some of the adherence to glycosphingolipids indicate reduced binding relative to BSA.

Effects of spirochetal strains and mutants on adherence to glycosphingolipids. Polyvinyl chloride plates were coated with GalCer, LacCer, GD1a, and GT1B at a concentration of 1 μg per well and blocked with PBS-BSA as described above. Spirochete strains B31, EBV−, ESP1, ESP1-EV, TI1, and TI1-EV were added, in triplicate, at concentrations of 1×10^6, 5×10^6, and 1×10^7. Plates were then incubated at 33°C for 4 h. Plates were washed, and adhered spirochetes were fixed with 10% formalin as described above. ELISA was performed as described above.

Inhibition of adherence. MAb H4610 (IgG2a), which recognized an epitope within the NH_2 terminus of OspB, was the gift of Thomas Schwan (Rocky Mountain Laboratories, Hamilton, Mont.). Spirochetes (all three strains and their respective mutants, at the same concentrations as those given above) were subjected to preincubation, coincubation, or pre- and coincubation with H4610 supernatant at an antibody/medium ratio of 1:5 followed by ELISA as described above. Spirochetes were preincubated with sugars (galactose, glucose, lactose, mannose, *N*-acetylgalactosamine, *N*-acetylglucosamine, and sialic acid) at final concentrations of 10, 20, 50, 100, and 200 mM for 3 h at 33°C prior to the adhesion assays and coincubated for the duration of the binding period.

Affinity-purified and recombinant OspA and OspB (0.5 μg/ml) were preincubated for 2 h at 33°C on glycosphingolipid-coated plates and then washed three times with PBS-BSA. Spirochete strain TI1 was then added at a concentration of 5×10^6 spirochetes per well to preincubated wells and control wells. After a 3-h incubation at 33°C and formalin fixation, the ELISA described earlier was performed.

Prior to all of the inhibition experiments, the effects of MAb H4610, sugars, and sialic acid concentration on the viability of the spirochetes for the duration of the incubation periods were measured by counting organisms before and after treatment and by a qualitative assessment of motility and viability using dark-field microscopy.

Statistics. Student's *t* tests and one-way analysis of variance (ANOVA) were done using In-Stat 2.01 from GraphPad Software, San Diego, Calif.

RESULTS

Adhesion of *B. burgdorferi* to glycosphingolipids. A very clear pattern of adhesion emerged from the binding assays (Fig. 1). *B. burgdorferi* bound to the short-sugar-chain glycosphingolipids GalCer, LacCer, and CerTri (Fig. 1). Optimal binding for GalCer was at 2 μg, for LacCer it was at 1 and 1.5 μg, and for CerTri it was at 0.5 μg per well. *B. burgdorferi* did not bind to the gangliosides bearing the Galβ1-3GalNac terminus, GM1 and GD1b, to gangliotetraosyl ceramide (asialo GM1 [AGM1]), or to globoside (globoseries) GL4 and ganglioside GM2, both bearing terminal GalNac. Adhesion to two gangliosides with terminal sialic acid, GD1a and GT1b, was highest at 1.5 and 0.5 μg per well, respectively. *B. burgdorferi* did not bind to GM3, which also carries a terminal sialic acid (Fig. 1). The same pattern of adhesion emerged when these experiments were repeated using radiolabeled organisms (data not shown). Because of these findings, all subsequent experiments were done with GalCer, LacCer, GD1a, and GT1b.

Binding to glycosphingolipids requires viable spirochetes. Only live *B. burgdorferi* organisms adhere to GalCer, LacCer, and CerTri (Table 2). A one-way ANOVA disclosed statisti-

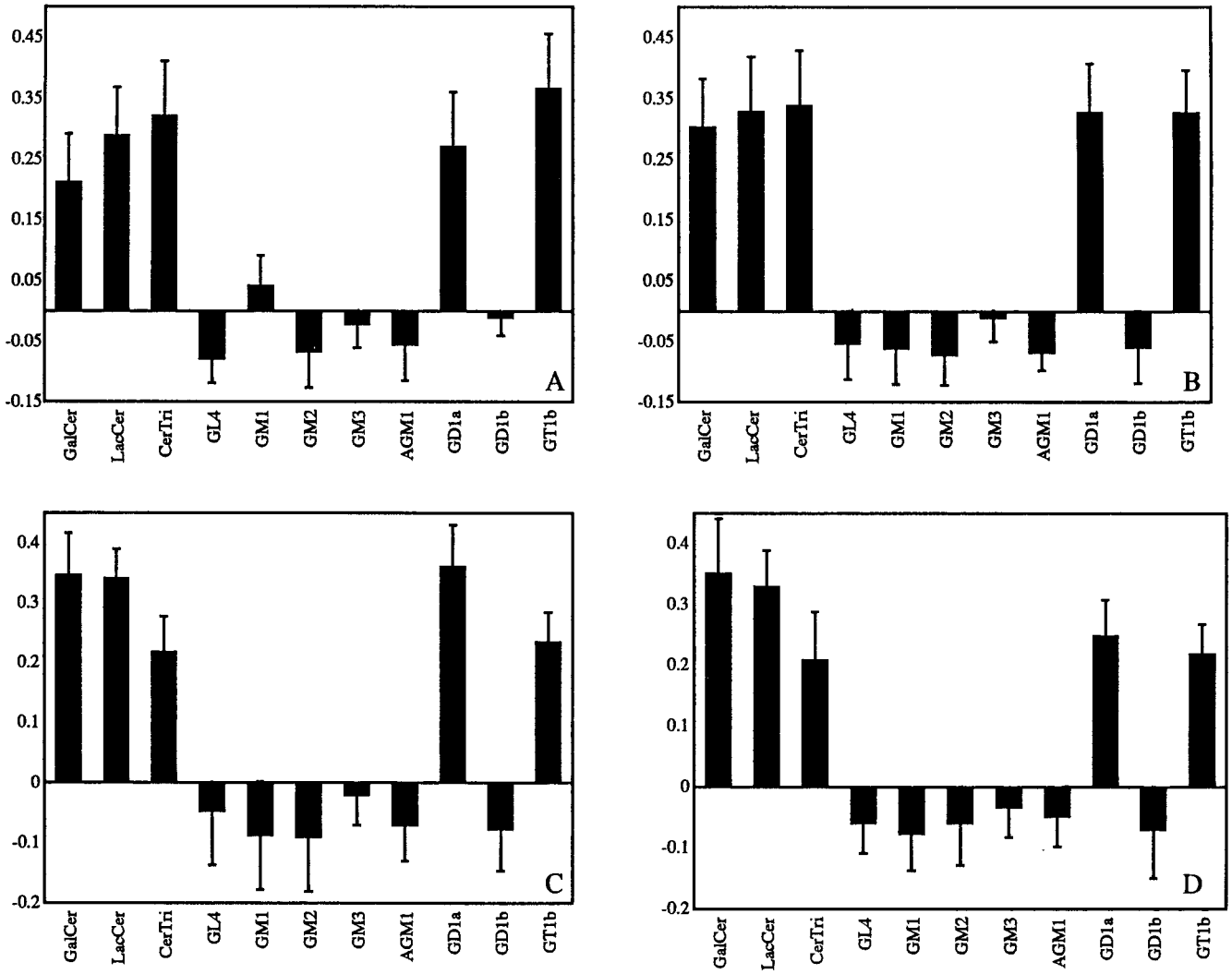

FIG. 1. Adhesion of *B. burgdorferi* (low-passage TI1) to glycosphingolipids. Bars represent mean optical densities for three wells for each concentration of each glycosphingolipid from which the mean optical density of control wells receiving BSA alone were subtracted ± the standard errors of the means. Glycosphingolipids at 0.5 μg per well (A), 1.0 μg per well (B), 1.5 μg per well (C), and 2.0 μg per well (D). These data are from a representative assay chosen from three identical experiments.

cally significant increases in binding of live organisms to all of the glycosphingolipids ($P < 0.001$) compared with the solvent control, except for binding to GT1b ($P < 0.05$). Binding of heat-killed and fixed organisms to GalCer and LacCer was not significantly different from binding to the solvent control. However, heat-killed and fixed organisms showed increased adherence ($P < 0.05$, compared with the solvent control) to CerTri and GT1b, and GD1a and GT1b, respectively. When adherence of heat-killed and formalin-fixed spirochetes was compared with that of live spirochetes, significant decreases were noted across all substrates (minimum $P < 0.05$).

Low-passage strains of *B. burgdorferi* show greater adherence to glycosphingolipids, and truncations in OspB do not result in decreased binding. ESP1 and TI1, both low-passage strains, adhered better to all four glycosphingolipids than did the high-passage strain B31 ($P < 0.05$). This is consistent with other observations (11, 17, 21, 23, 28, 44) on adherence by this species. We selected three wild-type strains and their respective OspB mutants (Fig. 2) to examine the role of OspB in adhesion to glycosphingolipids. The *ospB* genes of the three strains and each respective mutant have been sequenced, and the amino acid composition and length of each truncated mutant OspB is known (Fig. 2).

A complex pattern of adhesion emerged from the experiments using the three strains and their respective mutants (Fig. 3). In general, the OspB mutant strains bound the glycosphingolipids as well as, or better than, the parent wild-type strains, in some cases significantly so (ESP1 mutant to LacCer and B31 mutant to GT1b [Fig. 3, $P < 0.05$]). This indicates that adhesion of *B. burgdorferi* to glycosphingolipids is not affected by truncation of OspB.

Adherence of *B. burgdorferi* to glycosphingolipids was not inhibited by a MAb to OspB, by competition with sugars, or by soluble Osp. MAb H4610, with an epitope within the amino terminus of OspB (a gift from Thomas Schwan), which recognized both rOspB (missing the first 17 amino acids) as well as all the mutants of B31 and ESP1 (missing the last 120 and 177 amino acids, respectively) by Western blot, did not inhibit binding of either live organisms or the purified OspB (data not shown). This MAb did not react with the TI1 mutant, which is

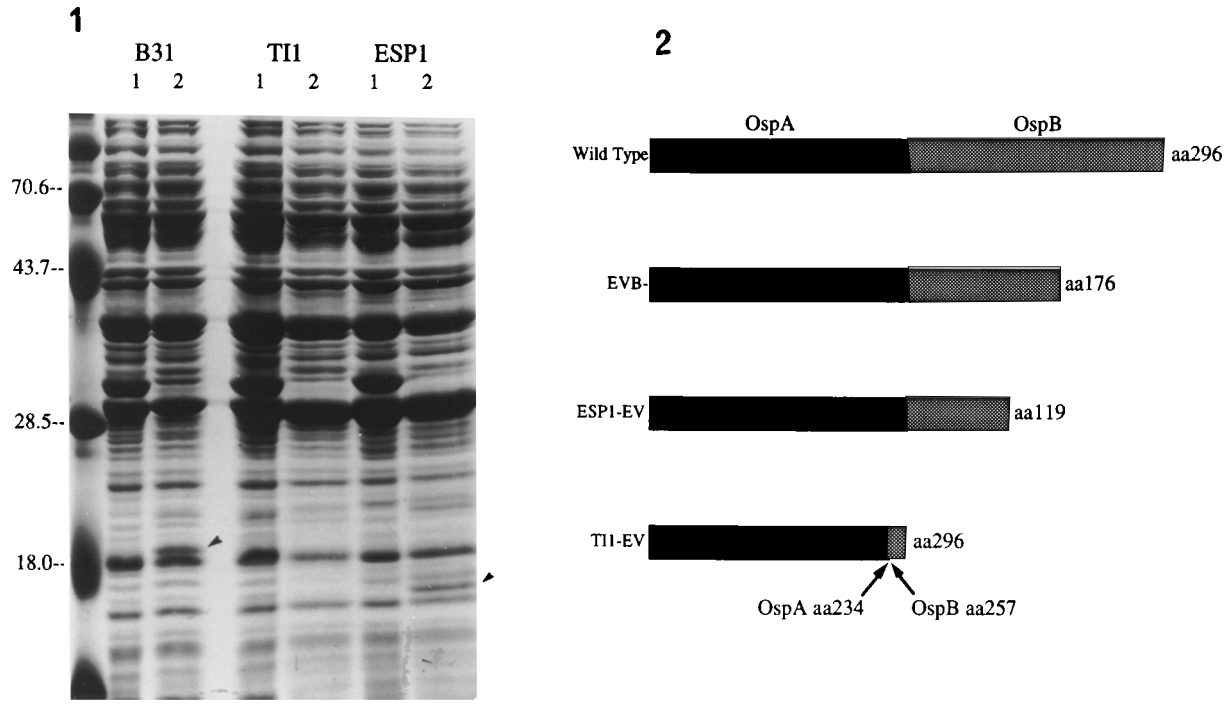

FIG. 2. (1) Coomassie blue-stained SDS-PAGE gel of wild type (lanes 1) and mutant (lanes 2) of three strains of *B. burgdorferi* used for assay of adhesion to glycosphingolipids. Arrowheads indicate the position of truncated OspB in EVB− and ESP1-EV. Molecular weight markers (in thousands) are shown on the left. (2) Schematic representation of mutations on *ospA-ospB* operon.

missing the amino terminus of OspB. From these experiments, it was possible to locate the epitope for H4610 within amino acids 17 to 118. MAb H4610 did not have a bactericidal effect by itself at the concentrations used.

Several concentrations (5, 12.5, 25, 50, 100, and 200 mM) of glucose, galactose, mannose, *n*-acetylglucosamine, *n*-acetylgalactosamine, and sialic acid pre- and coincubated with *B. burgdorferi* (strain TI1) failed to inhibit binding to any of the four glycosphingolipids. Preincubation of glycosphingolipid-coated plates with affinity-purified (Fig. 4) and recombinant OspA (0.5 μg/ml) did not result in decreased spirochetal adhesion. Preincubation with affinity-purified (Fig. 4) and recombinant OspB at the same concentrations resulted in significantly reduced binding to LacCer (36 and 38%, respectively; $P < 0.05$).

DISCUSSION

B. burgdorferi bound to three neutral glycosphingolipids, GalCer, LacCer, and CerTri, and to gangliosides GD1a and GT1b. The adherence of GalCer has been previously demonstrated in connection with the binding of *B. burgdorferi* to Schwann cells (24) and has been confirmed here with other strains of this organism. The binding of *B. burgdorferi* to LacCer places this organism within the large group of bacteria and fungi which have affinities for this glycosphingolipid (29–31, 41, 42). Neither galactose nor lactose at various concentrations pre- and coincubated with these organisms was able to inhibit binding to either GalCer or LacCer. This is in agreement with earlier observations made with other bacteria which bind LacCer (30, 31, 42). It has been proposed that the ceramide chemical composition (present in both GalCer and LacCer) provides the entire glycosphingolipid molecule with different energy levels (31). The determinant which accounts for these differences is the level of hydroxylation of the ceramide portion of the molecule (30, 31, 42). Along these lines, we were able to demonstrate the preference of *B. burgdorferi* for GalCer preparations with higher hydroxylation levels of the ceramide backbone (24), so it is possible that this may extend to the binding of LacCer by this organism.

B. burgdorferi bound to gangliosides GD1a and GT1b but not at all to globoside, asialo GM1, and/or gangliosides GM1, GM2, GM3, and GD1b. Gangliosides GD1a and GT1b share terminal sialic acids as does GM3 (to which *B. burgdorferi* did not bind). While adherence to the neutral glycosphingolipids could be nearly totally eliminated by heat killing and/or formalin fixation of the organisms, the same procedures reduced binding to GD1a and GT1b by 50 to 88%, suggesting a differ-

TABLE 2. Effects of heat killing and formalin fixation on adherence of *B. burgdorferi* to glycosphingolipids (percent decrease as compared with live organisms)

Glycosphingolipid	OD (mean ± SD)[a]		
	Live	Heat killed	Formalin fixed
Solvent	0.067 ± 0.01	0.049 ± 0.03 (27)	0.059 ± 0.01 (12)
GalCer	0.512 ± 0.04[a]	0.016 ± 0.01 (97)[b]	0.005 ± 0.01 (99)[b]
LacCer	0.610 ± 0.06[a]	0.064 ± 0.01 (90)[b]	0.059 ± 0.01 (90)[b]
CerTri	0.685 ± 0.04[a]	0.100 ± 0.02 (89)[a,b]	0.071 ± 0.05 (92)[b]
GD1a	0.498 ± 0.12[a]	0.060 ± 0.01 (88)[b]	0.160 ± 0.05 (68)[a,b]
GT1b	0.287 ± 0.07[a]	0.117 ± 0.05 (59)[a,b]	0.143 ± 0.03 (50)[a,b]

[a] Values are mean optical densities ± standard deviations for spirochetal adherence as determined by ELISA; the percent decrease in adherence is shown in parentheses. This assay used 3×10^6 organisms per well with various glycosphingolipids coated at 1 μg per well. Level of significance by one-way ANOVA within columns (vertical): [a]$P < 0.05$ compared with value for solvent. Level of significance by one-way ANOVA within rows (horizontal): [b]$P < 0.05$ compared with value for live.

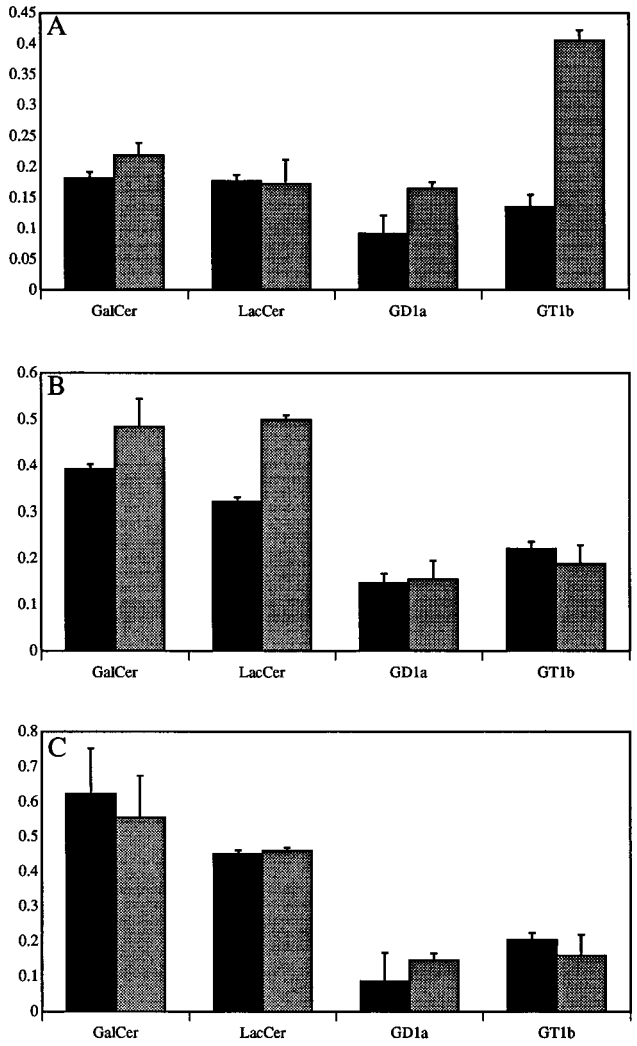

FIG. 3. Adhesion of three *B. burgdorferi* strains and their respective OspB mutants (5×10^6 spirochetes per well) to galactocerebroside, lactosylceramide, GD1a, and GT1b. Bars represent mean optical densities for three wells for each spirochete strain, from which the mean optical density of control wells was subtracted ± the standard errors of the means. (A) B31 (solid bars) and EVB− (hatched bars); (B) ESP1 (solid bars) and ESP1-EV (hatched bars); (C) TI1 (solid bars) and TI1-EV (hatched bars). These results are derived from a single representative experiment.

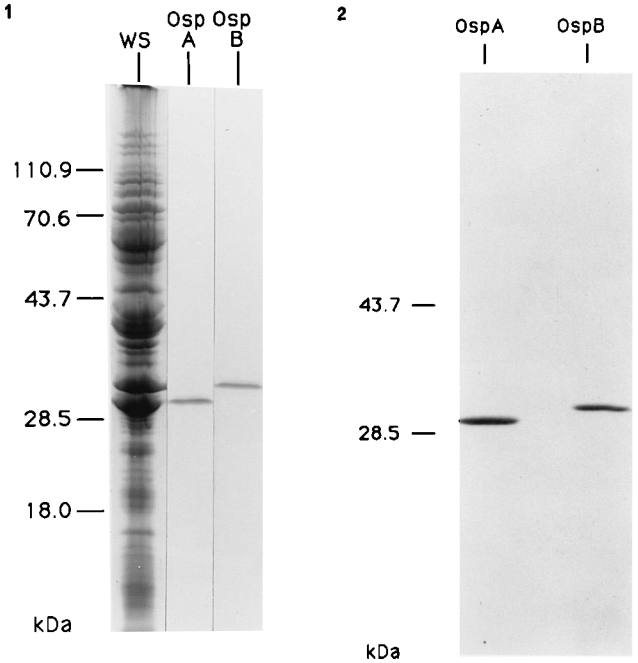

FIG. 4. (1) Coomassie blue-stained SDS-PAGE gel of affinity-purified OspA and OspB, with whole-cell sonicate (WS) used for comparison. Molecular weight markers (in thousands) are shown on the left. (2) Autoradiograph of affinity-purified OspA and OspB from *B. burgdorferi* biosynthetically radiolabeled with [9,10^{-3}H(*N*)]palmitic acid. Molecular weight markers in thousands are shown on the left.

ent pathway for adhesion (Table 2). The possibility that the terminal sialic acid could be the receptor for binding was enhanced by the fact that the organisms did not bind to GM1 (Table 1; Figure 1), which shares the same carbohydrate sequence as GD1a minus the terminal sialic acid. Various concentrations of sialic acid pre- and coincubated with *B. burgdorferi* failed to inhibit adhesion to GD1a and GT1b. These negative results, as well as the fact that *B. burgdorferi* did not bind to GM3 (also with a terminal sialic acid, Table 1), suggest that the presence of a terminal sialic acid is not sufficient for adhesion. Nonetheless, that *B. burgdorferi* did not bind to other long-chain neutral glycosphingolipids and to four acidic gangliosides suggests that this organism can discern among individual gangliosides of the ganglioseries which share an internal lactose group. In this regard, the conformation of the terminal sialic acid may be important for binding so that its soluble form cannot interfere with the adhesion process.

Glycosphingolipids are found in a large variety of cells, although their distribution by type of cell varies considerably, from reasonably specific (GalCer) to nearly ubiquitous (LacCer) (30, 31, 43). Thus, the binding of *B. burgdorferi* to neutral glycosphingolipids and to two acidic gangliosides is consistent with the nonspecificity shown by this organism in adhesion to cells (3, 16, 17, 21, 23, 24, 33, 44, 45). Gangliosides in particular are known to be cellular receptors for binding of many bacteria (30, 31) and bacterial toxins (19). Further bacterium-cell interactions could be possible through binding to gangliosides, as these molecules can be involved in cellular signal transduction (43).

The outer surface of *B. burgdorferi* has not been explored in depth. Freeze fracture electron microscopy shows a paucity of outer surface molecules in this organism (38). Although by definition the Osps (outer surface proteins) would have an external surface location, a case has been made for a periplasmic location as well for OspA and OspB (9). Nonetheless, immunogold probes as well as the ability to agglutinate *B. burgdorferi* with MAbs to OspA and OspB indicate that at least a portion of these molecules must be on the surface (1, 14). Therefore, OspA and OspB, because of their abundance, surface exposure, and a high positive charge (4, 6), can be considered prime candidates for being bacterial ligands for glycosphingolipids.

A Fab fragment of a MAb to OspA inhibited the adhesion of *B. burgdorferi* to endothelium, indicating a possible role for this lipoprotein in adhesion to these cells (16). Our earlier attempts at inhibiting adhesion to glycosphingolipids with whole MAbs to OspA and their Fab fragments were not successful (14, 15). Likewise, attempts at inhibiting adhesion with MAbs to epitopes within the carboxy terminus of OspB resulted in damage to the organisms and in the production of escape variants

(mutants) (14). The availability of a MAb (H4610) to an epitope in the NH_2 terminus of OspB and the availability of OspB mutants with truncations in the carboxy termini permitted an assessment of the contributions of each region of this molecule to adhesion to glycosphingolipids. MAb H4610 did not inhibit binding, and the mutants bound as well or better than the parent wild types. In addition, preincubation of substrates with affinity-purified and recombinant Osp did not result in decreased adhesion of organisms to most of the substrates used (except for LacCer). These data, interpreted collectively, do not suggest a role for OspA and OspB in adhesion to glycosphingolipids.

However, the approach taken here as well as in other studies to examine potential receptors may need to be reevaluated. The Osp could be complexed to other spirochetal components in their native state (26). Of the six Osps known (A to F) (6, 20, 35, 36), four are positively charged molecules (OspA, -B, -C, and -E), which could influence the net charge and polarity of the entire organism, which in turn could provide for electrostatic or ionic interactions with negatively charged substrates such as acidic gangliosides or proteoglycans (28) and with the polar heads of neutral glycosphingolipids.

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REFERENCES

1. **Barbour, A. G., S. L. Tessier, and W. J. Todd.** 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. Infect. Immun. **41:**795–804.
2. **Benach, J. L., E. M. Bosler, J. P. Hanrahan, J. L. Coleman, G. S. Habicht, T. F. Bast, D. J. Cameron, J. L. Ziegler, A. G. Barbour, W. Burgdorfer, R. Edelman, and R. A. Kaslow.** 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N. Engl. J. Med. **308:**740–742.
3. **Benach, J. L., J. L. Coleman, J. C. Garcia-Monco, and P. C. Deponte.** 1988. Biological activity of *Borrelia burgdorferi* antigens. Ann. N.Y. Acad. Sci. **539:**115–125.
4. **Benach, J. L., J. L. Coleman, and M. G. Golightly.** 1988. A murine IgM monoclonal antibody binds an antigenic determinant in outer surface protein A, an immunodominant basic protein of the Lyme disease spirochete. J. Immunol. **140:**265–272.
5. **Benach, J. L., H. B. Fleit, G. S. Habicht, J. L. Coleman, E. M. Bosler, and B. P. Lane.** 1984. Interactions of phagocytes with the Lyme disease spirochete: role of the FC receptor. J. Infect. Dis. **150:**497–507.
6. **Bergström, S., V. G. Bundoc, and A. G. Barbour.** 1989. Molecular analysis of linear plasmid encoded major surface proteins, OspA and OspB, of the Lyme disease spirochaete *Borrelia burgdorferi*. Mol. Microbiol. **3:**479–486.
7. **Bock, K., M. E. Breimer, A. Brignole, G. C. Hansson, K. A. Karlsson, G. Larson, H. Leffler, B. E. Samuelson, N. Strömberg, C. Svanborg-Edén, and J. Thurin.** 1985. Specificity of binding of a strain of uropathogenic *Escherichia coli* to Gal(α1-4)Gal containing glycosphingolipids. J. Biol. Chem. **260:**8545–8551.
8. **Brandt, M. E., B. S. Riley, J. D. Radolf, and M. V. Norgard.** 1990. Immunogenic integral membrane proteins of *Borrelia burgdorferi* are lipoproteins. Infect. Immun. **58:**983–991.
9. **Brusca, J. S., A. W. McDowall, M. V. Norgard, and J. D. Radolf.** 1991. Localization of outer surface proteins A and B in both the outer membrane and intracellular compartments of *Borrelia burgdorferi*. J. Bacteriol. **173:** 8004–8008.
10. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick-borne spirochetosis? Science **216:** 1317–1319.
11. **Coburn, J., J. M. Leong, and J. K. Erban.** 1993. Integrin $\alpha_{IIb}\beta_3$ mediates binding of the Lyme disease agent *Borrelia burgdorferi* to human platelets. Proc. Natl. Acad. Sci. USA **90:**7059–7063.
12. **Coleman, J. L., and J. L. Benach.** 1989. Identification and characterization of an endoflagellar antigen of *Borrelia burgdorferi*. J. Clin. Invest. **84:**322–330.
13. **Coleman, J. L., and J. L. Benach.** 1992. Characterization of antigenic determinants of *Borrelia burgdorferi* shared by other bacteria. J. Infect. Dis. **165:** 658–666.
14. **Coleman, J. L., R. C. Rogers, and J. L. Benach.** 1992. Selection of an escape variant of *Borrelia burgdorferi* by use of bactericidal monoclonal antibodies to OspB. Infect. Immun. **60:**3098–3104.
15. **Coleman, J. L., R. C. Rogers, P. A. Rosa, and J. L. Benach.** 1994. Variations in the OspB gene of *Borrelia burgdorferi* result in differences in monoclonal antibody reactivity and in production of escape variants. Infect. Immun. **62:**303–307.
16. **Comstock, L. E., E. Fikrig, R. J. Shoberg, R. A. Flavell, and D. D. Thomas.** 1993. A monoclonal antibody to OspA inhibits association of *Borrelia burgdorferi* with human endothelial cells. Infect. Immun. **61:**423–431.
17. **Comstock, L. E., and D. D. Thomas.** 1989. Penetration of endothelial cell monolayers by *Borrelia burgdorferi*. Infect. Immun. **57:**1626–1628.
18. **Dunn, J. J., B. N. Lade, and A. G. Barbour.** 1990. Outer surface protein (OspA) from the Lyme disease spirochete, *Borrelia burgdorferi*: high level expression and purification of a soluble recombinant form of OspA. Protein Exp. Purif. **1:**159–168.
19. **Fishman, P. H.** 1982. Role of membrane gangliosides in the binding and action of bacterial toxins. J. Membr. Biol. **69:**85–97.
20. **Fuchs, R., S. Jauris, F. Lottspeich, V. Preac-Mursic, B. Wilske, and E. Soutschek.** 1992. Molecular analysis and expression of a *Borrelia burgdorferi* gene encoding a 22kDa protein (pC) in *Escherichia coli*. Mol. Microbiol. **6:**503–509.
21. **Galbe, J. L., E. Guy, J. M. Zapatero, E. I. B. Peerschke, and J. L. Benach.** 1993. Vascular clearance of *Borrelia burgdorferi* in rats. Microb. Pathog. **14:**187–201.
22. **Garcia-Monco, J. C., J. L. Coleman, J. L. Galbe, A. Szczepanski, J. L. Benach, B. Fernandez Villar, C. Hughes, and R. C. Johnson.** 1991. Caracterizacion de una cepa española de *Borrelia burgdorferi*, el agente causal de la enfermedad de Lyme. Med. Clin. **98:**89–93.
23. **Garcia-Monco, J. C., B. Fernandez Villar, and J. L. Benach.** 1989. Adherence of the Lyme disease spirochete to glial cells and cells of glial origin. J. Infect. Dis. **160:**497–506.
24. **Garcia-Monco, J. C., B. Fernandez Villar, R. C. Rogers, A. Szczepanski, C. M. Wheeler, and J. L. Benach.** 1992. *Borrelia burgdorferi* and other related spirochetes bind to galactocerebroside. Neurology **42:**1341–1348.
25. **Garcia-Monco, J. C., C. M. Wheeler, J. L. Benach, R. A. Furie, S. A. Lukehart, G. Stanek, and A. C. Steere.** 1993. Reactivity of neuroborreliosis patients (Lyme disease) to cardiolipin and gangliosides. J. Neurol. Sci. **117:** 206–214.
26. **Gondolf, K., S. R. Batsford, and A. Vogt.** 1990. Isolation of an outer membrane protein complex from *Borrelia burgdorferi* by n-butanol extraction and high-performance ion-exchange chromatography. J. Chromatogr. **521:**325–334.
27. **Hazlett, L. D., S. Masinick, R. Barrett, and K. Rosol.** 1993. Evidence for asialo GM1 as a corneal glycolipid receptor for *Pseudomonas aeruginosa* adhesion. Infect. Immun. **61:**5164–5173.
28. **Isaacs, R. D.** 1994. *Borrelia burgdorferi* bind to epithelial cell proteoglycans. J. Clin. Invest. **93:**809–819.
29. **Jimenez-Lucho, V., V. Ginsburg, and H. C. Krivan.** 1990. *Cryptococcus neoformans*, *Candida albicans*, and other fungi bind specifically to the glycosphingolipid lactosylceramide (Galβ1-4Glcβ1-1Cer), a possible adhesion receptor for yeasts. Infect. Immun. **58:**2085–2090.
30. **Karlsson, K. A.** 1989. Animal glycosphingolipids as membrane attachment sites for bacteria. Annu. Rev. Biochem. **58:**309–350.
31. **Karlsson, K. A., J. Ångström, J. Bergström, and B. Lanne.** 1992. Microbial interaction with animal cell surface carbohydrates. APMIS Suppl. **100:**71–83.
32. **Krivan, H. C., D. D. Roberts, and V. Ginsburg.** 1988. Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNac(β1-4)Gal found in some glycolipids. Proc. Natl. Acad. Sci. USA **85:** 6157–6161.
33. **Kurtti, T. J., U. G. Munderloh, D. E. Krueger, R. C. Johnson, and T. G. Schwan.** 1993. Adhesion to and invasion of cultured tick (Acarina: Ixodidae) cells by *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) and maintenance of infectivity. J. Med. Entomol. **30:**586–596.
34. **Laemmli, U. K., and M. Favre.** 1973. Maturation of the head of bacteriophage T4. I. DNA packing events. J. Mol. Biol. **80:**575–599.
35. **Lam, T. T., T. K. Nguyen, R. R. Montgomery, F. S. Kantor, E. Fikrig, and R. A. Flavell.** 1994. Outer surface proteins E and F of *Borrelia burgdorferi*, the agent of Lyme disease. Infect. Immun. **62:**290–298.
36. **Norris, S. J., C. J. Carter, J. K. Howell, and A. G. Barbour.** 1992. Low-passage-associated proteins of *Borrelia burgdorferi* B31: characterization and molecular cloning of OspD, a surface-exposed, plasmid-encoded lipoprotein. Infect. Immun. **60:**4662–4672.
37. **Palestini, P., M. Masserini, A. Fiorilli, E. Calappi, and G. Tettamanti.** 1991. Evidence for nonrandom distribution of GD1a ganglioside in rabbit brain microsomal membranes. J. Neurochem. **57:**748–753.
38. **Radolf, J. D., K. W. Bourell, D. R. Akins, J. S. Brusca, and M. V. Norgard.** 1994. Analysis of *Borrelia burgdorferi* membrane architechture by freeze-fracture electron microscopy. J. Bacteriol. **176:**21–31.
39. **Rosa, P. A., T. Schwan, and D. Hogan.** 1992. Recombination between genes encoding major outer surface proteins A and B of *Borrelia burgdorferi*. Mol. Microbiol. **6:**3031–3040.
40. **Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista.** 1983. The

spirochetal etiology of Lyme disease. N. Engl. J. Med. **308:**733–740.
41. **Strömberg, N., C. Deal, G. Nyberg, S. Normark, M. So, and K. A. Karlsson.** 1988. Identification of carbohydrate structures that are possible receptors for *Neisseria gonorrhoeae*. Proc. Natl. Acad. Sci. USA **85:**4902–4906.
42. **Strömberg, N., and K. A. Karlsson.** 1990. Characterization of the binding of *Propionibacterium granulosum* to glycosphingolipids adsorbed on surfaces. J. Biol. Chem. **265:**11244–11250.
43. **Stults, C. L. M., C. C. Sweeley, and B. A. Macher.** 1989. Glycosphingolipids: structure, biological source, and properties. Methods Enzymol. **179:**167–214.
44. **Szczepanski, A., M. B. Furie, J. L. Benach, B. P. Lane, and H. B. Fleit.** 1990. Interaction between *Borrelia burgdorferi* and endothelium in vitro. J. Clin. Invest. **85:**1637–1647.
45. **Thomas, D. D., and L. E. Comstock.** 1989. Interaction of Lyme disease spirochetes with cultured eucaryotic cells. Infect. Immun. **57:**1324–1326.