

Interactions between Extracellular *Borrelia burgdorferi* Proteins and Non-*Borrelia*-Directed Immunoglobulin M Antibodies

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Previous work showed that outer surface protein A (OspA) and OspB of *Borrelia burgdorferi* may occur within an extracellular multiprotein complex, which was resolved by electrophoresis as an 83-kDa major extracellular protein band. To characterize the 83-kDa band, we sequenced the N terminus of the predominant peptide in the band and examined the interaction between the associated proteins. Peptide sequence and amino acid composition comparisons showed identity with the heavy chain of immunoglobulin M (IgM). Reduction sensitivity experiments and the recognition of the band by antibodies specific for rabbit μ chain indicated that the multiprotein complex contained pentameric IgM. Immunoelectron microscopy showed that anti- μ chain antibodies and monoclonal antibodies to OspA and OspB bound to extracellular amorphous material surrounding cells. Furthermore, the Osps coprecipitated with either nonspecific polyclonal rabbit IgM antibodies or with murine monoclonal anti-human serum albumin IgM antibodies, using insoluble anti- μ chain antibody conjugates. Although the apparent 83-kDa complex was stable under conditions of chelation and concentrated salts, it was disrupted by treatment with neuraminidase. These results indicate that extracellular *B. burgdorferi* proteins, including OspA and OspB, interact with IgM. The association is apparently not a classic antibody-antigen interaction but may result from other mechanisms.

Infection with the spirochete *Borrelia burgdorferi* causes the acute and chronic manifestations of Lyme borreliosis (7). Despite considerable work on humoral and cell-mediated responses to infection, which has recently been reviewed (24, 25), the pathogenic mechanisms that contribute to chronic disease induced by this spirochete remain obscure. It is evident that *B. burgdorferi* is susceptible to complement-mediated cytolysis and phagocytic clearance (24, 25). Furthermore, most, if not all, uncompromised mammalian hosts develop a significant humoral response to infection (24, 25). However, the persistent infections documented in humans and animal models indicate that immune clearance is either rare or nonexistent (24, 25). Several studies have demonstrated passive protection by antibodies and active protection by vaccination in animal models (14, 24, 25). Such protection is apparently transient, requiring immunization during a limited period prior to infectious challenge.

Little evidence suggests that antigenic variation contributes significantly to transient protection and persistent infection (24, 25). Although antigenic differences among various isolates have been documented (3, 23-25), dramatic antigenic changes during chronic infection have not been reported. Furthermore, no evidence has been described for antigenic shifts or gene rearrangements in *B. burgdorferi* similar to those believed to function as immune evasion mechanisms in the relapsing fever agent *B. hermsii* (2). Such observations have led to suggestions that *B. burgdorferi* persistent infections are maintained by spirochetes that become sequestered from immune effectors.

Recent studies using an antigen capture and detection assay demonstrated aggregates of extracellular *B. burgdorferi* antigens in fluids and tissues from infected arthropod and mammalian hosts (13). The assay system utilized polyclonal F(ab')₂ fragments generated against extracellular vesicle

concentrates to capture antigens, which were subsequently labeled and detected by using polyclonal immunoglobulin G (IgG) antibodies specific for *B. burgdorferi*. These antibodies were raised against an 83-kDa major extracellular protein band, but reacted primarily with outer surface protein A (OspA) and OspB from geographically diverse isolates. The assay enabled consistent detection of the aggregates in samples from which spirochetes were rarely observed, suggesting that the aggregates were constantly circulating or that the aggregates were deposited by motile spirochetes and persisted in situ. The study also showed that the antigen aggregates could be recovered from culture supernatants along with extracellular membrane vesicles and that the aggregates may be derived from s-layer material. However, the apparent relationship between the Osps and the 83-kDa band was not pursued beyond demonstrating the reactivity of anti-major extracellular protein IgG antibodies (13). To investigate the relationship between these vesicle-associated proteins, we characterized the 83-kDa band with respect to its composition and its sensitivity to reducing agents and glycosidases. In this report, we describe an interaction between certain *B. burgdorferi* surface proteins and non-*Borrelia*-directed IgM antibodies.

MATERIALS AND METHODS

Bacteria. *B. burgdorferi* Sh-2-82 was maintained at 36°C in BSK II medium as previously described (1). The medium was prepared freshly and was not heat inactivated. Serum-free cultures of this strain were also provided by Andrew Szczepanski.

Recovery of whole cells and extracellular materials. Whole cells, extracellular vesicles, and vesicle-associated materials were recovered from cultures by differential and sucrose density gradient centrifugation according to previously published procedures (10, 15). Culture supernatants were filter sterilized by using nitrocellulose membranes with 0.22- μ m

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porosity (Nalge Co., Rochester, N.Y.) prior to recovery of extracellular products. Pellets containing extracellular products were either resuspended in appropriate buffers for electron microscopy, enzymatic treatment, or electrophoresis (see below) or stored at -20°C .

Electrophoresis. Whole cells and extracellular products were solubilized and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using the discontinuous buffer system of Laemmli (17), with published modifications (16). The separated proteins were either stained with Coomassie brilliant blue (CBB) and destained with deionized water or electroblotted onto nitrocellulose membranes as previously described (5) or onto polyvinylidene difluoride (PVDF) membranes (Millipore Corp., Bedford, Mass.), according to the manufacturer's recommendations. For experiments assessing the effects of reduction on protein migration, extracellular concentrates or gel segments containing specific proteins were also extracted in Laemmli buffer lacking 2-mercaptoethanol (17).

Amino acid analysis. Electrophoretically separated *B. burgdorferi* vesicle-associated proteins were electroblotted onto PVDF membranes as described above and stained with CBB. Excised segments of PVDF membranes containing the 83-kDa band were hydrolyzed for 2 h at 160°C in the vapor phase of 6 N HCl and 2% (vol/vol) liquified phenol. Membrane segments were then rinsed with 40% acetonitrile and then 0.5% trifluoroacetic acid in 40% acetonitrile to recover the hydrolyzed amino acids. Phenylthiocarbonyl-amino acid analysis was performed on a model 420A derivatizer coupled to a model 130A separation system (Applied Biosystems, Inc., Foster City, Calif.). The determined amino acid composition was compared with the compositions of all 35- to 90-kDa proteins contained in the Martinsrieder Institut für Proteinsequenzen, MIPSX version 17.0 protein data base (Martinsrieder, Germany), using program A3COMP (9) to identify similar compositions.

Protein sequencing. Excised segments of PVDF membranes containing the 83-kDa band were also used for protein sequencing. Sequencing was enabled by removal of a pyroglutamate residue from the N terminus. A PVDF segment containing 19 μg of protein was pretreated with 0.5% polyvinylpyrrolidone-40 (Sigma Chemical Co., St. Louis, Mo.) in 100 mM acetic acid for 30 min at 37°C . Following 10 water washes, the segments were incubated under nitrogen for 5 h at 4°C with 5 μg of calf liver pyroglutamate aminopeptidase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) in buffer containing 100 mM dibasic sodium phosphate, 10 mM disodium EDTA, 5 mM dithiothreitol, and 5% (vol/vol) glycerol (pH 8.0). Incubation was continued for 16 h at 30°C , after adding an additional 5 μg of enzyme, and the treated segment was rinsed twice with water. Sequencing was performed in the gas phase on a model 477A protein sequencer with an on-line model 120A analyzer (Applied Biosystems, Inc.) and model 2600 chromatography software (Nelson Analytical, Cupertino, Calif.).

Antibodies and immunoblot analysis. Supernatants from hybridomas 5332 and 5TS, which recognize OspA and OspB, respectively (3, 4), were graciously provided by Tom Schwan (Rocky Mountain Laboratories, Hamilton, Mont.). Purified murine monoclonal IgM was a gift from William Knowles (Miles Laboratories, West Haven, Conn.). Goat anti-rabbit μ chain antiserum and horseradish peroxidase (HRP)-conjugated goat anti-rabbit μ chain antibodies were purchased from Organon Teknica-Cappel (Durham, N.C.). Goat anti-mouse μ chain antibodies, protein G, and protein A-HRP were purchased from Sigma Chemical Co. Colloidal

gold conjugates of protein G and anti- μ chain antibodies were produced for this study by previously published procedures (21). These antibodies were used to probe electroblotted whole cell and extracellular antigens, and resulting immune complexes were detected by peroxidase staining as previously described (5), using appropriate immune complex-peroxidase conjugates.

Immunoelectron microscopy. Parlodion-coated electron microscopy grids were activated with anti-vesicle F(ab')_2 fragments as previously described (13). Whole cells, extracellular concentrates, or uninoculated medium was incubated with the grids for 20 min at room temperature and then washed, probed with antibodies, and labeled with appropriate colloidal gold conjugates as previously described (13, 21). After labeling, the grids were washed briefly with 0.25 M ammonium acetate, negatively stained with 0.5% ammonium molybdate (pH 6.5), and observed at 75 kV with an HU-11E-1 transmission electron microscope (Hitachi, Ltd., Tokyo, Japan).

Immunoprecipitation. Components of filter-sterilized culture supernatants were immunoprecipitated from solution by using 30-nm colloidal gold conjugates of either anti-rabbit or anti-(mouse μ chain) antibodies. Five milliliters of supernatant from standard BSK II cultures or from serum-free cultures containing or lacking murine monoclonal IgM antibodies were incubated at 25°C for 1 h with 1 ml of conjugate containing approximately 30 μg of anti- μ chain antibodies. Resulting immune complexes were recovered by centrifugation at $38,500 \times g$ in a TLA 45 rotor (Beckman Instruments, Palo Alto, Calif.). Pellets were washed twice by resuspending in sterile Dulbecco's phosphate-buffered saline and recovering by centrifugation. Samples of uninoculated medium incubated with the conjugate and samples of filtered supernatant incubated with stabilizing buffer lacking the conjugate were also prepared as described above. Washed pellets were then extracted in Laemmli buffer for subsequent electrophoretic and immunoblot analyses.

Neuraminidase treatment. For neuraminidase treatment of the 83-kDa band, SDS-polyacrylamide gels were stained with CBB in water, and the 83-kDa band was excised. Excised gel segments were macerated in 20 μl of buffer containing 20 mM sodium cacodylate (pH 7.2), 10 mM CaCl_2 , 200 mM sucrose, and 1 μg each of the proteinase inhibitors aprotinin, bestatin, and E-64 (Boehringer Mannheim Biochemicals), which also contained or lacked 0.1 U of neuraminidase (United States Biochemical Corp., Cleveland, Ohio). After incubation for 16 h at 4°C , the mixtures were extracted with 20 μl of Laemmli buffer containing 4% SDS, and the liquid phase was retained for subsequent electrophoresis. The second gels were electroblotted onto nitrocellulose and probed with either a mixture of monoclonal antibodies 5332 and 5TS and then protein A-HRP or anti-(rabbit μ chain)-HRP. Antibody binding was visualized by peroxidase staining.

Accession number. The N-terminal amino acid sequence determined for the 83-kDa peptide was entered in the National Biomedical Research Foundation sequence library. The accession number is A37272.

RESULTS

Previous work with *B. burgdorferi* extracellular vesicle preparations demonstrated a highly concentrated protein band with an electrophoretic migration of 83 kDa and an uncharacterized relationship with OspA and OspB (13). To identify the predominant peptide present in the band, we

TABLE 1. Amino acid composition of the 83-kDa band^a

Amino acid	Mol%	Amino acid	Mol%
Ala.....	6.25	Lys.....	4.26
Arg.....	2.94	Met.....	0.59
Asx.....	9.46	Phe.....	4.37
Glx.....	10.75	Pro.....	6.94
Gly.....	7.43	Ser.....	14.80
His.....	1.22	Thr.....	8.39
Ile.....	2.71	Tyr.....	2.72
Leu.....	7.83	Val.....	9.35

^a Cys and Trp were not determined.

electroblotted vesicle-associated proteins onto PVDF membranes, excised the 83-kDa band, and determined both the amino acid composition (Table 1) and the N-terminal amino acid sequence (Fig. 1). Figure 1 shows the 83-kDa band within a CBB-stained SDS-12.5% polyacrylamide gel and the sequence obtained for residues 1 to 14 (upper sequence). Residue 1 was pyroglutamate, residue 2 could not be identified, and residue 10 was tentatively identified as serine. An example of the results of a sequence homology search is also shown (lower sequence, Fig. 1). We compared this partial sequence with the National Biomedical Research Foundation protein sequence bank using the FASTA program (20) and found that up to 11 of 12 aligned residues were identical with N-terminal residues of Ig heavy chain from a variety of mammals. Furthermore, a comparison of the amino acid composition with proteins in the Martinsrieder Institut für Proteinsequenzen data base showed the greatest identity with IgM.

To determine whether the sequenced peptide was derived from intact monomeric or oligomeric antibody molecules, we examined the effects of reduction on the migration of the 83-kDa band (Fig. 2). Figure 2 shows the pertinent portions of a CBB-stained gel (Fig. 2A) and an immunoblot (Fig. 2B). When extracted with Laemmli buffer lacking 2-mercaptoethanol, extracellular preparations lacked the concentrated

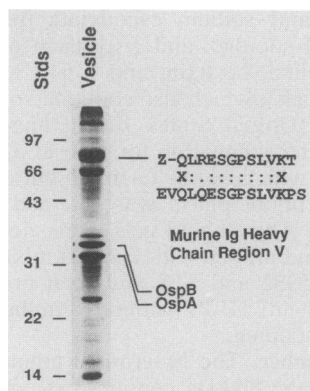


FIG. 1. Electrophoretic position and N-terminal sequence of a major component of the 83-kDa major extracellular protein band. Extracellular concentrates were separated by SDS-PAGE, electroblotted onto PVDF membranes, and stained with CBB. The 83-kDa band was excised and sequenced. Residues 3 to 14 (upper sequence) were then screened for sequence homology with the National Biomedical Research Foundation data base. The partial sequence (NBRF A37272) shared 91.9% identity with the N terminus of Ig heavy chain from a mouse (lower sequence) (NBRF A02098). The region of identity is delineated between two Xs. Stds, molecular mass standards in kilodaltons.

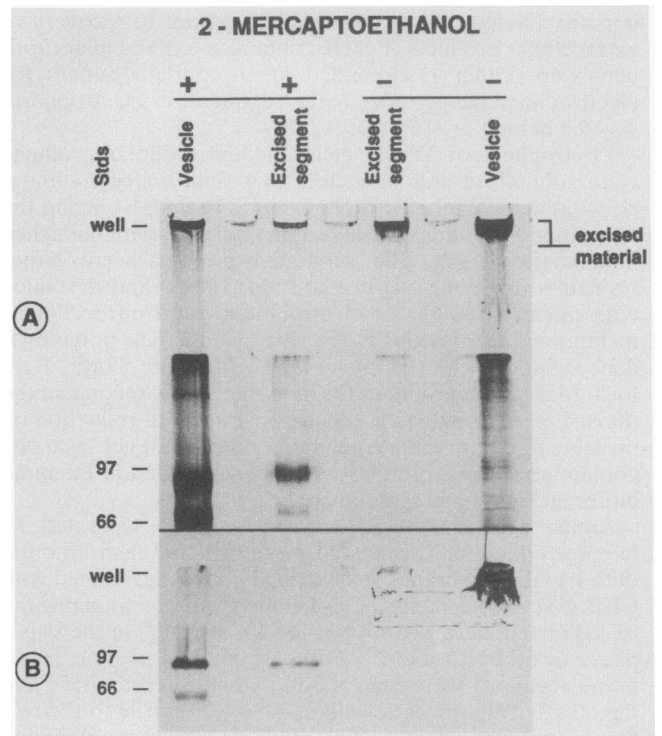


FIG. 2. Effect of reduction on the electrophoretic migration of the 83-kDa vesicle-associated protein band. Extracellular concentrates were extracted in Laemmli buffer containing or lacking 2-mercaptoethanol and then separated by SDS-PAGE. In preparations lacking 2-mercaptoethanol, the 83-kDa band was not detected, but an accumulation of protein was evident in the stacking gel, just under the loading well (A). When this accumulated protein was excised and reextracted with or without 2-mercaptoethanol, the unreduced extract remained in the stacking gel, whereas the reduced extract migrated at 83 kDa. When an immunoblot of the same preparations was probed with anti-rabbit μ chain antibody-peroxidase conjugates and visualized by peroxidase staining, both the 83-kDa band and the accumulated protein in the stacking gel were stained (B). Stds, molecular mass standards in kilodaltons.

staining at 83 kDa, and protein accumulated in the stacking gel, just below the loading well (Fig. 2A). When we excised the gel segment containing the accumulated protein and reextracted the segment with Laemmli buffer containing 2-mercaptoethanol, the 83-kDa band was restored. Without reduction, the reextracted protein again accumulated in the stacking gel. We also probed an electroblot containing these preparations with anti- μ chain-HRP and then visualized the antibody binding by peroxidase staining (Fig. 2B). Both the 83-kDa band and the unreduced protein which accumulated in the stacking gel reacted strongly with this antibody. An additional labeled band at 57 kDa, which is characteristic of Ig heavy chain, was detected in the reduced extracellular protein preparation. Light-chain molecules were also detected at approximately 30 kDa in reduced preparations by using heavy- and light-chain-specific reagents (data not shown).

Since previous work had demonstrated that rabbits immunized with the 83-kDa band produced antibodies that reacted with OspA and OspB, we used immunoelectron microscopy and immunoprecipitation (Fig. 3) to determine whether these proteins interact within *in vitro* cultures. The figure is a composite of micrographs and segments of an SDS-poly-

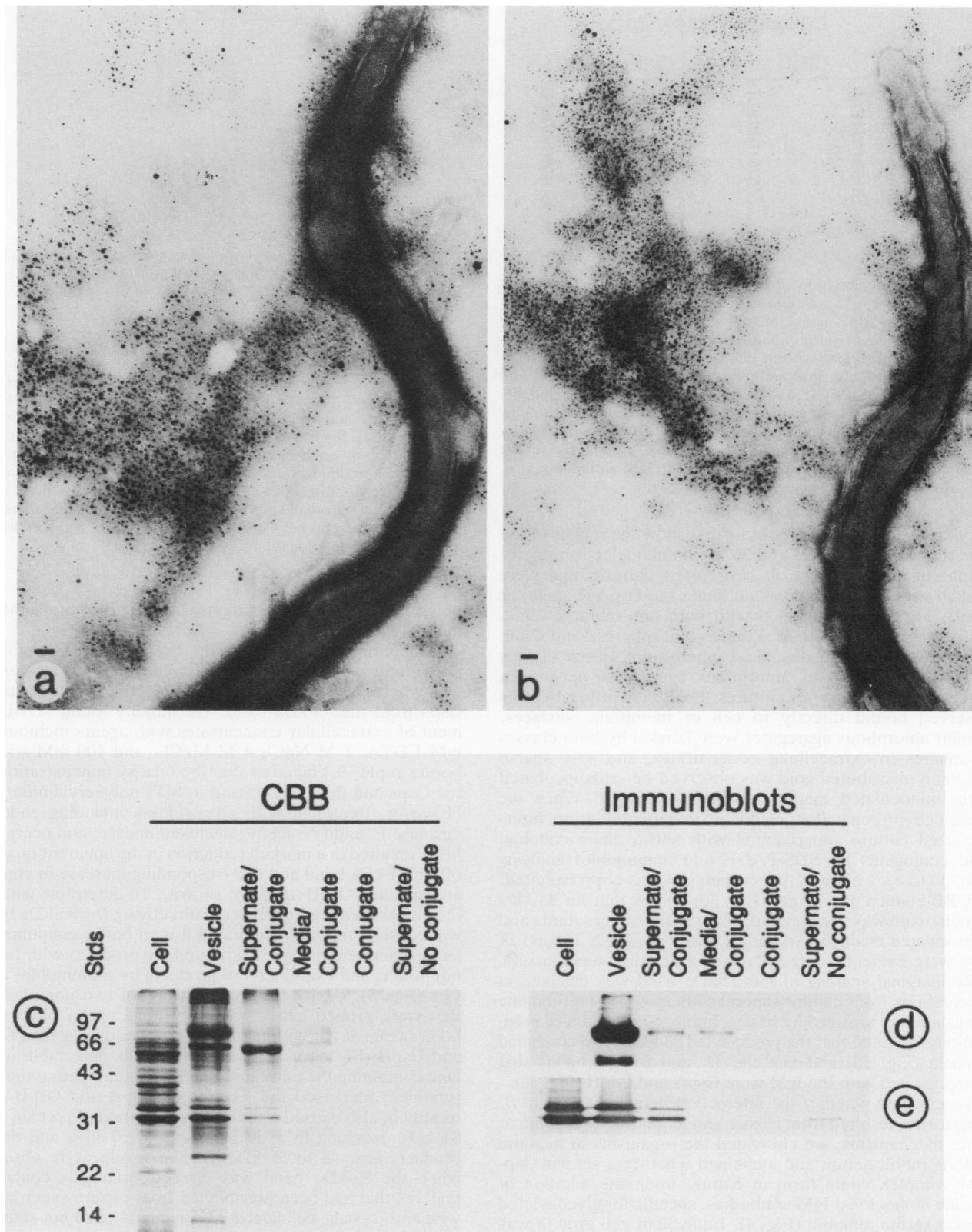


FIG. 3. Extracellular association of epitopes for IgM, OspA, and OspB in cultures of *B. burgdorferi*. Mid-log-phase spirochetes, extracellular concentrates, and uninoculated medium were adsorbed to antivesicle F(ab')₂-activated electron microscope grids. The grids were then incubated with monoclonal antibodies directed against OspA (a) or OspB (b) and labeled with colloidal gold conjugates of both protein G (7 to 8 nm) and anti-rabbit μ chain antibodies (15 nm). Electron microscopy showed that both classes of particles bound primarily to extracellular amorphous material that surrounded cells and extracellular vesicles. Relatively little gold was deposited directly on cell surfaces or membranes. Grids incubated with uninoculated medium contained minimal, randomly dispersed gold spheres (data not shown). We also precipitated antigens from culture supernatants using anti-(rabbit μ chain)-colloidal gold conjugates. A CBB-stained 12.5% gel (c) showed that a band migrating at 83 kDa was recovered from uninoculated medium. Bands at 83, 34, 32, 24, and 14 kDa were observed in precipitants from culture supernatants. Immunoblots with specific antibodies indicated that the bands at 83, 34, and 32 kDa contained μ chain (d), OspB, and OspA (e), respectively. Stds, molecular mass standards in kilodaltons; scale bars, 100 nm.

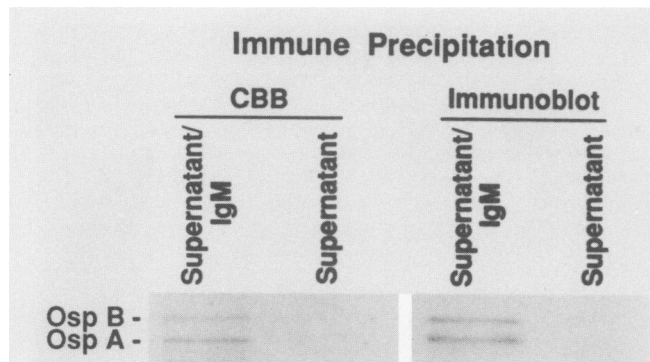


FIG. 4. Coprecipitation of *B. burgdorferi* proteins and nonspecific monoclonal IgM antibodies. Serum-free cultures were supplemented with murine monoclonal IgM antibodies directed against glycosylated human serum albumin or left unsupplemented. Antigens present in filter-sterilized culture supernatants were precipitated with anti-mouse IgM antibody–colloidal gold conjugates and subjected to SDS-PAGE and immunoblot analysis. Precipitants from cultures supplemented with IgM antibodies contained proteins at 32 and 34 kDa, which were identified as OspA and OspB by reactivity with monoclonal antibodies. These proteins were not detected in precipitants from cultures lacking IgM antibodies.

acrylamide gel and immunoblots that show the results of the experiments. Figures 3a and 3b are immunoelectron micrographs of mid-log-phase *B. burgdorferi* cultures that were probed with IgG monoclonal antibodies to OspA (Fig. 3a) or OspB (Fig. 3b) and labeled with both anti-rabbit μ chain antibodies, conjugated to 15-nm gold spheres, and 7-nm gold-protein G conjugates. Gold spheres of both size classes were concentrated on amorphous aggregates of antigen present in *B. burgdorferi* cultures. Relatively little label was observed bound directly to cell or membrane surfaces. Similar amorphous aggregates were labeled by both classes of spheres in extracellular concentrates, and only sparse randomly distributed gold was observed on grids incubated with uninoculated medium (data not shown). When we examined proteins that could be precipitated from filter-sterilized culture supernatants with anti- μ chain–colloidal gold conjugates by SDS-PAGE and immunoblot analysis (Fig. 3c to e), we found that certain proteins coprecipitated. A CBB-stained 12.5% gel (Fig. 3c) shows that an 83-kDa protein band was precipitated from culture supernatants and uninoculated medium. Additional bands at 34, 32, 24, and 14 kDa were evident in precipitants from culture supernatants. Only marginal staining of the 32-kDa band was detected in preparations from culture supernatants in which the immune conjugate was replaced by buffer. Immunoblots of these preparations indicated that the precipitated 83-kDa band contained μ chain (Fig. 3d) and that the 32- and 34-kDa bands that coprecipitated with the IgM were OspA and OspB (Fig. 3e).

To evaluate whether the interaction between IgM and *B. burgdorferi* resulted from classic antigen-antibody binding or other mechanisms, we cultivated the organisms in medium lacking rabbit serum and examined whether a similar Osp-IgM complex could form in culture upon the addition of murine monoclonal IgM antibodies, specific for glycosylated human serum albumin (Fig. 4). Equivalent cell growth was observed in cultures containing or lacking monoclonal IgM. Using colloidal gold conjugates of anti-(mouse μ -chain)-specific antibodies to precipitate antigens from filter-sterilized supernatants of such cultures, OspA and OspB coprecipitated with IgM, but the Osps were not detected in

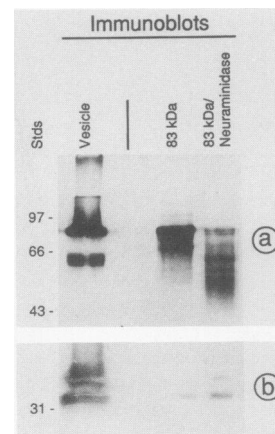


FIG. 5. Effect of neuraminidase treatment on the 83-kDa vesicle-associated protein band. Extracellular proteins were separated by SDS-PAGE, and the 83-kDa bands were excised from the gel. Gel segments were macerated and incubated with buffer containing neuraminidase and proteinase inhibitors or buffer and the inhibitors only (see text). Such mixtures were then reextracted and examined by immunoblot analysis, using anti- μ chain antibodies (a) and monoclonal antibodies to OspA and OspB (b). Neuraminidase treatment resulted in both an electrophoretic shift in the μ chain from 83 kDa to products at 57 kDa and 45 to 55 kDa and increased labeling and detection of OspA and OspB proteins. Stds, molecular mass standards in kilodaltons.

precipitants from supernatants lacking the supplemental monoclonal antibodies.

To identify components in the excised 83-kDa band that might induce anti-Osp rabbit antibodies (13), we also examined whether we could dissociate detectable quantities of the Osps from the 83-kDa band. We initially found that treatment of extracellular concentrates with agents including 50 mM EDTA, 1 M NaCl, 4 M MgCl₂, and 100 mM sodium borate at pH 10.4 failed to alter the relative concentrations of the Osps and the 83-kDa band in SDS-polyacrylamide gels. However, treatment with glycosidases including endoglycosidase F, endo-*N*-acetylgalactosaminidase, and neuraminidase resulted in a marked reduction in the apparent quantity of the 83-kDa band and a corresponding increase in staining at OspA and OspB (data not shown). To determine whether the glycosidase treatment acted directly on the 83-kDa band, we excised the band, incubated it with buffer containing or lacking neuraminidase, reextracted the products with Laemmli buffer, and examined the products by immunoblot analysis (Fig. 5). Figure 5 shows portions of two immunoblots that were probed either with polyclonal anti- μ chain antibodies (Fig. 5a) or with monoclonal antibodies against OspA and OspB (Fig. 5b). Although OspA was detectable in the lane containing the untreated 83-kDa protein, neuraminidase treatment increased the labeling of OspA and OspB. The treatment also caused a shift in the migration of μ chain from 83 kDa, resulting in a distinct band at 57 kDa and diffuse products near 45 to 55 kDa. Similar results were observed when the 83-kDa band was excised from gels containing material that had been precipitated from culture supernatants with anti- μ chain–colloidal gold conjugates (data not shown).

DISCUSSION

By analyzing the components of a predominant extracellular vesicle-associated 83-kDa protein band, we found that

certain extracellular *B. burgdorferi* proteins interact with the μ chain of IgM. The interaction produces a multiprotein complex that is abundant in *B. burgdorferi* cultures and resembles structurally similar material that has previously been detected in fluids and tissues from infected hosts (13). Furthermore, our results suggest that the complex can occur within in vitro cultures, incorporating non-*B. burgdorferi*-directed IgM antibodies.

In this study, we found that the N-terminal peptide sequence of the major component of the band corresponded to mammalian Ig μ chain. Therefore, we conclude that this extracellular band is distinct from the 83-kDa cellular protein described by LeFebvre and others (18). Failure to codetect the Osp or other sequences could result from either a low relative quantity of such proteins in the band or N-terminal acylation of the mature Osps (6), or both. Similarly, since the amino acid composition of this band reflected IgM, we presume that the relative molar ratios of other proteins in the band are low.

Clearly, the 83-kDa band in extracellular concentrates contains a mixture of proteins. Two lines of evidence indicate that quantities of OspA and OspB are retained within an electrophoretic protein band at 83 kDa. In a previous study (13) and in subsequent experiments (12), rabbits immunized with proteins contained within this band consistently developed a pronounced IgG antibody response to the Osps. This study found that OspA and OspB could also be detected in gels and on immunoblots after excision, neuraminidase treatment, and reextraction of the 83-kDa band. Inconsistent binding by anti-Osp monoclonal and polyclonal antibodies (12, 13) to the 83-kDa band and minimal intrinsic labeling of proteins migrating at 83 kDa suggest that relatively little *B. burgdorferi* protein is present in the band. However, the relative quantities of μ chain and Osp molecules present in the band have not been determined. Such a determination will likely involve several variables including the possibility of additional *B. burgdorferi* or eukaryotic proteins in the band, the possible involvement of 1 to 10 heavy-chain molecules per IgM pentamer, the in vitro growth rate of *B. burgdorferi*, and the biochemical nature of the Osp-IgM interactions.

The presence of IgM in extracellular concentrates was confirmed by antibody recognition and reduction sensitivity. Electrophoretic retardation of the unreduced protein greatly exceeded the predicted value of 150 to 180 kDa for monomeric Ig molecules. Furthermore, the reduction-sensitive bands reacted with μ -chain-specific antiserum but not with γ -chain-specific reagents including protein A, protein G, and anti-rabbit IgG antibodies (data not shown). Immunoelectron microscopy and immunoprecipitations demonstrated that the apparent interaction between IgM and the Osps occurs extracellularly. When cultured spirochetes and extracellular concentrates were probed with antibodies specific for either OspA or OspB and μ chain, and the antibodies were labeled with different-sized gold spheres, the majority of both size classes of spheres was concentrated on extracellular amorphous material. These results were consistent with previous immunoelectron microscopic findings of amorphous aggregates of extracellular antigens, including OspA and OspB, in fluids and tissues of infected hosts (13). Relatively little of the labeled material was retained directly on cell or membrane surfaces, suggesting that surface-exposed epitopes, recognized by monoclonal antibodies 5332 and 5TS (3, 4), are located primarily on exported forms of OspA and OspB. This observation was supported by immunoprecipitation results with the anti- μ chain antibody-

colloidal gold conjugates. Precipitation of filter-sterilized culture supernatants with this conjugate resulted in the specific and simultaneous recovery of IgM, OspA, and OspB.

The nature of the interactions between the proteins in the extracellular complex remains undetermined. However, several lines of evidence suggest that factors other than specific immune complexes are involved. Although treatment with sodium borate at pH 10.4 is known to dissociate circulating immune complexes involving *B. burgdorferi* antigens (22), such treatment had no apparent effect on our recovery of the Osp proteins or of proteins comigrating at 83 kDa in extracellular concentrates (data not shown). Furthermore, the complex is consistently observed in cultures supplemented with pooled normal rabbit serum, and we found that monoclonal IgM antibodies directed against an unrelated eukaryotic protein could interact with *B. burgdorferi* proteins in a similar manner. Our experimental results suggest that at least two interactions between IgM and the Osps occur. The results of immunoprecipitations demonstrated that significant quantities of Osp-IgM complexes are dissociated by extraction with SDS and 2-mercaptoethanol. Although further dissociation of Osps and IgM antibodies present in the 83-kDa band did not occur after chelation with 50 mM EDTA, and salt treatments including 1 M NaCl and 4 M MgCl₂ (data not shown), an additional fraction of the Osps was released from the 83-kDa band by neuraminidase treatment and reextraction with Laemmli buffer. This suggests that the Osps or other associated *B. burgdorferi* proteins can interact with reduced μ chains and that sialic acid residues contribute to the retention of Osps within the 83-kDa band in gels. Whether this enzymatic disruption of the protein complex indicates a covalent or other stable interaction between the constituents remains to be determined. Neuraminidase treatment also caused a marked decrease in the apparent mass of the majority of μ -chain molecules migrating at 83 kDa. Since μ -chain molecules can exhibit apparent molecular masses of between 57 and 84 kDa depending on degree of glycosylation and whether the IgM is secreted or incorporated on the surface of B lymphocytes, and since the effect of *B. burgdorferi* peptides on the migration of μ chains is unclear, such a shift in migration could result from the dissociation of Osps, deglycosylation, or both. Furthermore, although proteinase inhibitors were included during neuraminidase treatments, we cannot rule out the possibility that proteolysis also contributed to the dissociation of Osps and μ chains. Whether the *B. burgdorferi* proteins bind directly to sialic acid or whether the neuraminidase treatment interrupts the interaction by a steric or conformational change in the IgM or spirochetal proteins remains unclear. The involvement of glycosylated μ chains in interactions between IgM and these extracellular proteins may, however, contribute to explanations for the affinity of certain lectins for extracellular *B. burgdorferi* antigens (11), the 83-kDa band (12), and other specific *B. burgdorferi* proteins (8, 19).

Previous work demonstrated that similar extracellular protein aggregates, which contain epitopes for OspA and OspB, apparently persist in a variety of fluids and tissues from hosts infected with *B. burgdorferi* (13), and preliminary results suggest that such aggregates also contain IgM antibodies (12). It is generally accepted that IgG-binding proteins A and G are virulence factors for staphylococci and streptococci, respectively. Therefore, the possibility that IgM-Osp complexes contribute to immune evasion by means such as camouflaging its surface or rendering humoral responses ineffective needs consideration. Furthermore, since autoimmune dysfunctions have been proposed as factors in Lyme borrelio-

sis (24, 25), the possibility that Osp-IgM complexes induce autoreactive antibodies should also be examined.

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