JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1994, p. 423–429
0095-1137/94/$04.00+0

Vol. 32, No. 2

Monoclonal Antibody to Native P39 Protein from *Borrelia burgdorferi*

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Received 3 August 1993/Returned for modification 21 October 1993/Accepted 5 November 1993

We have produced, by using a sonicate of *Borrelia burgdorferi*, a monoclonal antibody (MAb), NYSP39H, that is specific for the P39 protein band. This MAb reacted with 13 isolates of *B. burgdorferi* but not with eight different spirochetes (four borrelias, two leptospiras, and two treponemas). Surface labeling of *B. burgdorferi* with biotin and subsequent treatment with Nonidet P-40 showed that P39 was not biotinylated but was extracted with Nonidet P-40, indicating that it is present within the outer membrane, but not on the surface of the spirochete. Immunoelectron microscopy revealed the immunogold probe primarily at the cytoplasmic membrane region of the spirochete. The MAb detected *B. burgdorferi* in the indirect fluorescent-antibody test only when the spirochetes from a culture or in a tick homogenate were fixed with polylysine and not with acetone. NYSP39H appears to be an appropriate probe for use in the specific detection of *B. burgdorferi*.

Borrelia burgdorferi, a member of the family *Spirochaetaceae*, is the causative agent of Lyme borreliosis (7), a disease which exhibits variable clinical manifestations (27). Cross-reacting epitopes among the members of this family are extensive (6 and references cited therein); as a result, serologic confirmation of Lyme disease in certain cases is unreliable (5). This is particularly true in patients with ill-defined clinical symptoms and with relatively low serum antibody titers to *B. burgdorferi*.

Similarly, the detection of *B. burgdorferi* by fluorescent-antibody microscopy with polyclonal antisera to *B. burgdorferi* is hindered by problems of specificity because of the presence in polyclonal antisera to *B. burgdorferi* of antibodies which react with common epitopes among members of the family *Spirochaetaceae* (19).

Simpson et al. (26) and Simpson et al. (25) have shown that a 39-kDa antigen (P39) is specific for *B. burgdorferi*. Polyclonal anti-recombinant P39 rabbit sera did not react in immunoblot studies with other *Borrelia* species. Immunoelectron microscopy with flagellin (41-kDa) as an antigen was negative with the anti-P39 sera, indicating that the 39-kDa antigen was distinct from the 41-kDa antigen. Although both antigen bands migrated very closely, they were separate entities and were not related to OspA or OspB. In addition, it was observed that P39 is conserved among North American and European isolates. Preliminary enzyme-linked immunosorbent assay (ELISA) studies (26), with P39 as the ligand, of sera from human Lyme borreliosis patients and of sera from experimentally and naturally inoculated animals suggest that P39 may be a useful marker for the specific serodiagnosis of Lyme borreliosis. Recently, however, Ma et al. (17) and Dressler et al. (10) showed that a number of syphilis sera reacted with what appears to be the P39 band from *B. burgdorferi*.

We have developed and characterized a monoclonal antibody (MAb) (NYSP39H) to the native P39 antigen by using whole *B. burgdorferi* as a source of antigen. NYSP39H is specific for *B. burgdorferi*, as shown by its lack of reactivity with eight different spirochetes, including *Treponema pallidum*, the causative agent of syphilis. It was also used to localize the P39 antigen in *B. burgdorferi*. NYSP39H can be used to detect the presence of *B. burgdorferi* in ticks and probably to isolate native P39.

MATERIALS AND METHODS

Antigens. The following *B. burgdorferi* isolates were used: ATCC 35210; isolate B31-H, which was obtained after 1,000 in vitro passages in BSK II medium (2) of the original Shelter Island isolate; HBH-1 and HBH-8 (low passage, i.e., one to five), collected from adult *Ixodes scapularis* ticks in the town of North Castle, Westchester County, NY, and obtained from Richard Falco (Westchester County Health Department, Valhalla, N.Y.); four Texas isolates obtained from Julia Rawlings (Texas Health Department, Austin); and five isolates obtained from Thomas Quan (Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colo.). Recombinant P39 antigen samples were obtained from Tom G. Schwan (Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Mont.) and from Brian Kiehl and Michael W. Andersen (General Biometrics Inc., San Diego, Calif.). For specificity studies, the following were used: *Borrelia hermsii* (ATCC 35209); *Borrelia parkeri*, *Borrelia coriaceae*, and *Borrelia turicatae*, obtained from Thomas Quan; *Treponema phagedenis* biotype Reiter, obtained from R. M. Smibert (Virginia Polytechnic Institute and State University, Blacksburg); *Leptospira icterohaemorrhagiae* RGA and *Leptospira canicola* Hond Utrecht IV, received (solubilized in Laemmli's buffer) from G. Baranton (Institut Pasteur, Paris, France); and *T. pallidum* obtained from K. Wicher (Wadsworth Center for Laboratories and Research, Albany, N.Y.). Each of the *Borrelia* species was grown in 750 ml of BSK II medium in multiple 1-liter flasks at 35°C. The inoculum was 10 ml of a 5-day growth culture. *B. burgdorferi* and *B. hermsii* were grown for 5 to 7 days, and the remaining

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three species were grown for 10 to 13 days. The *Borrelia* cultures were then processed as described previously (12). *T. phagedenis* was grown in multiple cultures of 100 ml of pepticase-yeast-glucose semisolid–Smibert's salt solution-serum–cocarboxylase medium (as described by R. M. Smibert [26a]). *T. phagedenis* was then processed as described for the borreliae.

Ticks. *I. scapularis* specimens were collected in the town of North Castle, Westchester County, N.Y., a Lyme disease-endemic area. They were washed with 70% ethanol and dissected by use of 26-gauge needles, and the midgut was removed, minced, and suspended in 10 mM Tris buffer (pH 7.5) containing 1 mM EDTA. The midgut homogenate was centrifuged at 13,000 × *g* for 5 min. The homogenates were used to detect the presence of *B. burgdorferi* in ticks by the indirect fluorescent-antibody (IFA) test (see below).

Production of hybridomas. Hybridomas were prepared as described by Rudofsky et al. (22). Lyophilized *B. burgdorferi* was suspended in phosphate-buffered saline (PBS) (0.4 mg/ml; equivalent to approximately 3×10^8 spirochetes), homogenized, and sonicated for 30 s. The suspension was then diluted with an equal volume of 4% aluminum hydroxide gel adjuvant or PBS. BALB/cByJ mice were immunized subcutaneously with 0.5 ml of the suspension in the adjuvant. The injections were repeated twice at weekly intervals with 0.5 ml of the suspension in PBS. At the end of this immunization schedule, serum specimens obtained were tested for antibodies to *B. burgdorferi* by an ELISA and by immunoblotting. A mouse with the highest titer was selected for hybridoma production. The spleen cell donor mouse was boosted with 0.5 ml of the suspension in PBS by the intraperitoneal route 3 days before the fusion. Spleen cells were fused with BALB/c P3 × 63-Ag8.653 myeloma cells by use of polyethylene glycol 1000. The spleen cell/myeloma cell ratio was approximately 5:1. Eight 96-cluster tissue-culture plates containing macrophage feeder layers were seeded with 1×10^6 to 2×10^6 cells per well). The cells were grown in hypoxanthine-aminopterin-thymidine medium containing 20% fetal calf serum and other additives. Clones were tested by the IFA test and the ELISA (see below). Both in vitro and in vivo propagation of the hybridoma lines was done according to standard procedures. The selected MAb was designated NYSP39H.

Polyclonal antisera. Polyclonal antisera were prepared as described previously (13). Rabbit antisera to each *Borrelia* species and to *T. phagedenis* were prepared by injecting each rabbit with 2 mg (dry weight; equivalent to approximately 1.5×10^9 organisms) of antigen suspended in 0.5 ml of aluminum hydroxide gel. The rabbits were boosted twice with 2 mg of antigen in PBS. At 4 weeks after the first injection, the rabbits were injected intramuscularly with ketamine hydrochloride (Ketaset; Veterinary Products, Bristol Laboratories, Syracuse, NY) and bled by cardiac puncture. Rabbit antisera to the recombinant P39 antigen and to the *Escherichia coli* carrier (obtained from T. Schwan) were also prepared as described above, with the exception that 0.2 ml of the recombinant or *E. coli* suspensions was mixed with 0.2 ml of the adjuvant.

Commercial antisera. All commercial unconjugated and conjugated anti-immunoglobulin sera were absorbed with a mixture of five borreliael antigens before use. This was done to eliminate immunofluorescence background reactions of 1+ to 2+, of ELISA optical density values of 0.2 to 0.3, or of faint bands in the immunoblot assay, which were often noticed with unabsorbed batches of commercial reagents. These background reactions were observed when these sera were reacted in the buffer control tests (NYSP39H was omitted from these tests). These antisera were diluted 1:20 in a suspension of PBS (pH 7.2) containing 0.5 mg (dry weight) each of *B. burgdorferi*, *B. parkeri*, *B. coriaceae*, *B. hermsii*, and *B. turicatae* per ml. The mixture was incubated at 37°C for 2 h and centrifuged at 7,000 × *g* for 30 min. The supernatant was filtered through a 0.2-μm-pore-size Millipore filter and further diluted according to the requirements of the test to be performed.

IFA test. Ten-well slides were treated with poly-L-lysine (21) by adding 20 μl of a 1:20 dilution in distilled water (dH_2O) to each well. The slides were incubated for 5 min at 25°C, rinsed by being dipped 10 times in dH_2O, and then allowed to air-dry for approximately 30 min. Two microliters of spirochetal suspension or tick homogenate (see above) was added to each well and allowed to dry at room temperature. Twenty microliters of mouse monoclonal ascites fluid, diluted 1:5 in buffer, was added to each well and incubated at 37°C for 60 min. Following incubation, the antibody was aspirated, and the slides were washed twice in PBS (pH 7.2) and rinsed 10 times in dH_2O.

To each well, 25 μl of a 1:30 dilution of fluorescein isothiocyanate-conjugated goat immunoglobulin G (IgG) (fraction) to mouse IgG (whole molecule) (Organon Teknika Corp., Durham, N.C.) was added, the slides were incubated at 37°C for 60 min and washed, and the stained spirochetes were observed microscopically.

ELISA. The ELISA with sonicated whole spirochetal antigens was performed as described previously (13). The ELISA with recombinant P39 antigen was performed as described in the insert supplied with the immunoWell P39 kit (General Biometrics), with modifications. To determine the class of immunoglobulin or the IgG subclass of the MAb, the MAb was first diluted 1:2 in the specimen diluent, further diluted 1:100 in the commercial blocking reagent, and incubated at 25°C for 30 min. The sample was added to the microtiter plates containing recombinant P39. The reaction mixture was incubated at 25°C for 2 h, and then the wells were washed three times with the wash buffer. The wells were incubated at 25°C for 1 h with conjugated goat anti-mouse IgG (whole molecule) (Organon Teknika), anti-mouse IgM (μ chain specific) (Organon Teknika), or anti-mouse IgG (γ chain specific) to each of the subclasses (Sigma Chemical Co., St. Louis, Mo.). All conjugated antisera were diluted 1:1,000. The wells were washed and incubated at 25°C for 1 h with horseradish peroxidase (HRP)-conjugated rabbit anti-goat IgG (Fc fragment specific) (Organon Teknika) diluted 1:1,000. The color was developed and color development was stopped with reagents supplied in the kit, and the optical density was read at 410 nm.

SDS gel electrophoresis and electroblotting. Sodium dodecyl sulfate (SDS) gel electrophoresis and electroblotting of whole spirochetal antigens were performed as described previously (12). SDS gel electrophoresis of recombinant-P39 antigen was performed with 4% stacking and 12.5% separating gels by use of the Mini-Protean II system (Bio-Rad Laboratories, Hercules, Calif.).

2-D gel electrophoresis. Two-dimensional (2-D) electrophoresis was carried out by the method of Hochstrasser et al. (14), with modifications. Lyophilized *B. burgdorferi* ATCC 35210 was suspended to 6 mg/ml, diluted with 1.0 ml of sample preparation solution D (1 g of SDS and 0.232 g of dithiothreitol in 10 ml of dH_2O), heated to 95°C, and then treated with 2.0 ml of solution E {0.1 g of dithiothreitol, 0.4 g of (CHAPS), 5.4 g of urea, and 0.5 ml of pH 3 to 10 ampholytes in 6.5 ml of dH_2O} to yield a final concentration

of 2 mg/ml. Thirty microliters of the sample was loaded onto isoelectric focusing gels. The isoelectric focusing gels contained 10 g of urea, 7.0 ml of dH_2O, and 2.5 ml of acrylamide-piperazine diacrylamide (30:0.8) (Bio-Rad). After the urea was dissolved, 0.8 ml of pH 5 to 7 ampholytes and 0.2 ml of pH 3 to 10 ampholytes (Bio-Rad) were added. The gels were focused at 200 V for 2 h, 500 V for 4 h, and 800 V for 18 h at room temperature with 0.85% phosphoric acid as the anolyte and 20 mM sodium hydroxide as the catholyte. Separation in the second dimension was carried out on SDS–12.5% polyacrylamide slab gels (20 cm by 20 cm by 1.5 mm) (Bio-Rad Protean II system) electrophoresed at 35 mA per gel until the dye front was about 0.5 in. (ca. 1.3 cm) from the bottom of the gel. Gels were electroblotted as described above or serially stained with Coomassie blue and silver stain (Bio-Rad silver Stain Plus). For pI determinations, 2-D SDS-polyacrylamide gel electrophoresis standards (Bio-Rad) were focused, electrophoresed, and stained as described above. Immunoblotting with NYSP39H was carried out as described above.

Immunoblotting. Immunoblotting was performed as described previously (12). The color was developed with 4-chloro-1-naphthol when the HRP-labeled probe was used or with fast red when the alkaline phosphatase-labeled probe was used. The subclass of NYSP39H was determined with unconjugated antisera to the immunoglobulin class or subclass and then probed as described above.

Biotin treatment of *B. burgdorferi*. *B. burgdorferi* grown for 6 days was divided into three aliquots. The spirochetes from each aliquot were harvested by centrifugation, and the pellets were suspended in Hanks' medium (Sigma Chemical Co.) and washed three times by microcentrifugation. One pellet was resuspended in 1 ml of Hanks' medium. The second pellet was resuspended in Hanks' medium and sonicated three times for 30 s each time. The third pellet was resuspended in 1 ml of a mixture of Hanks' medium and solubilizing buffer (12) without mercaptoethanol (volume/volume), treated at 56°C for 20 min, and then dialyzed with Hanks' medium. Each of the three aliquots was biotinylated with 5 μl of NHS-LC-Biotin (Pierce, Rockford, Ill.) in dimethyl sulfoxide (2.5%) for 30 min at 25°C. The biotinylation was terminated by dialysis with several changes of normal saline at 4°C.

The concentration of the biotinylated material was measured by use of the Pierce protein assay as described in the package insert. Each of the portions of material was adjusted with saline to 0.2 to 0.26 mg of protein per ml, solubilized, electrophoresed, and electroblotted on nitrocellulose paper.

A two-step immunoblot procedure was performed. For step 1, the biotinylated antigen bands were probed with avidin-HRP (Bio-Rad), and the color was developed. For step 2, the antigen bands were further reacted with NYSP39H and probed with alkaline phosphatase-conjugated sheep anti-mouse IgG (heavy and light chain specific) (Organon Teknika), and the color was developed.

NP-40 extraction. *B. burgdorferi* was surface labeled with NHS-LC-Biotin as described above. The labeled spirochetes were then treated with 0.2% Nonidet P-40 (NP-40) detergent at 37°C for 30 min to extract the outer membrane. Following NP-40 extraction, the suspension was centrifuged at 10,000 × *g* for 30 min to separate the extracted proteins (supernatant) from the remainder of the spirochetes (pellet). The protein contents of the supernatant and pellet were determined as described above, and each was adjusted with PBS (pH 7.2) to 0.2 to 0.26 mg of protein per ml, treated with solubilizing buffer, electrophoresed, and electroblotted on a nitrocellulose membrane. The electroblotted antigens were immunoblotted with the MAb or blotted with avidin-HRP as described above.

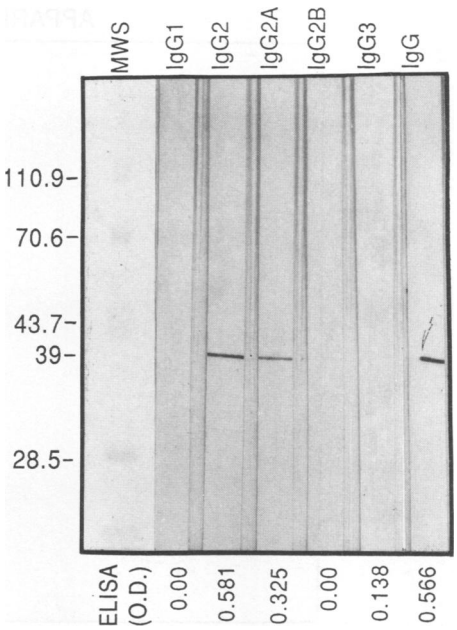

FIG. 1. Immunoblots of NYSP39H with *B. burgdorferi* ATCC 35210 for determination of its IgG subclass. Lanes were probed with antisera to IgG and to IgG subclasses (1, 2, 2A, 2B, and 3). The ELISA titer with each probe is shown at the bottom of each lane. MWS, molecular weight standards, in thousands. P39 had an M_r equivalent to 39,000. O.D., optical density.

Immunoelectron microscopy. Fixation and labeling of cells for immunoelectron microscopy were performed essentially as described previously (13). In brief, cells were fixed with 1% formaldehyde–0.2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 20 min at 25°C. After being washed with buffer, cells were dehydrated in a graded ethanol series and embedded in Epon-Araldite. Thin sections were retrieved on nickel grids, glow discharged, and floated on bovine serum albumin-Tris buffer (9) supplemented with 5% normal rabbit serum. The sections were treated at room temperature for 30 min with 1:10 to 1:100 dilutions (in bovine serum albumin-Tris buffer supplemented with 1% rabbit serum) of primary antibodies, which were either polyclonal mouse anti-*B. burgdorferi* antibodies, MAb NYSP39H, or normal mouse ascites fluid as a control. After being washed with bovine serum albumin-Tris buffer, the sections were exposed to a 1:20 dilution of rabbit anti-mouse IgG bound to 5-nm colloidal gold (Janssen, Piscataway, N.J.) and then stained with uranyl and lead salts prior to being viewed in the electron microscope.

RESULTS

Isotyping of NYSP39H (Fig. 1) by the ELISA showed that it belonged to the IgG2A subclass. This finding was also confirmed by immunoblotting (Fig. 1). The reactivity in the immunoblot test further indicated that NYSP39H was directed to an epitope present in one band with a relative mobility of 39 kDa. Also, immunoblotting of NYSP39H with the *B. burgdorferi* antigen that had been electrophoresed in a 2-D gel and electroblotted showed that it reacted with an

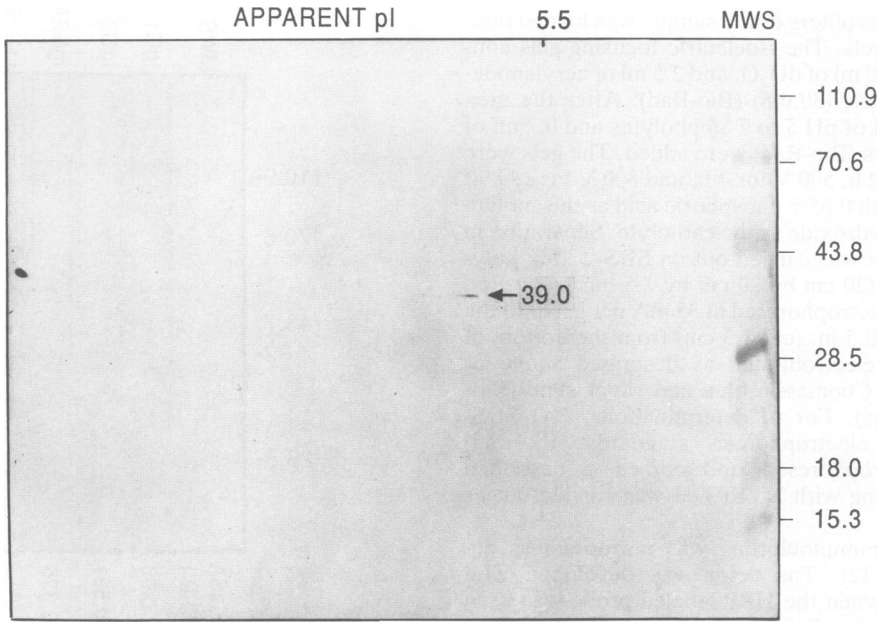

FIG. 2. Immunoblot of NYSP39H with 2-D-electrophoresed and electroblotted *B. burgdorferi* ATCC 35210. MWS, molecular weight standards, in thousands.

epitope from one antigen band with an apparent pI of 5.5 (Fig. 2). NYSP39H did not react with the other borrelial species or with members of the other genera of the family *Spirochaetaceae*; however, NYSP39H did react with the 13 isolates of *B. burgdorferi*, 4 of which are shown in Fig. 3. In Fig. 4, panel I shows the reactivity with avidin-HRP of four spirochetal preparations. Panel II, the immunospecific control, shows the presence of P39 in all four preparations. In each panel, lanes a, b, c, and d represent, respectively, biotinylated whole spirochetes sonicated and biotinylated spirochetes, solubilized and biotinylated spirochetes, and nonbiotinylated *B. burgdorferi*. P39 from whole biotinylated *B. burgdorferi* did not react with the avidin-HRP probe. In contrast, P39 from the fragmented or solubilized spirochetes reacted with avidin-HRP, indicating that P39 from these preparations was coupled to biotin. Optimal biotinylation appeared to have occurred with the latter preparation.

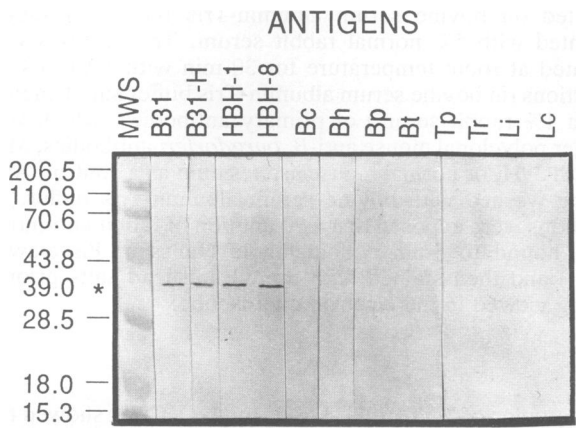

FIG. 3. Homologous seroreactivity of NYSP39H with antigens from four *B. burgdorferi* isolates: ATCC 35210 (B31), B31-H, HBH-1, and HBH-8. Also shown is the heterologous seroreactivity of P39 with the following antigens: *B. coriaceae* (Bc), *B. hermsii* (Bh), *B. parkeri* (Bp), *B. turicatae* (Bt), *T. pallidum* (Tp), *T. phagedenis* biotype Reiter (Tr), *L. icterohaemorragiae* (Li), and *L. canicola* (Lc). MWS, molecular weight standards, in thousands.

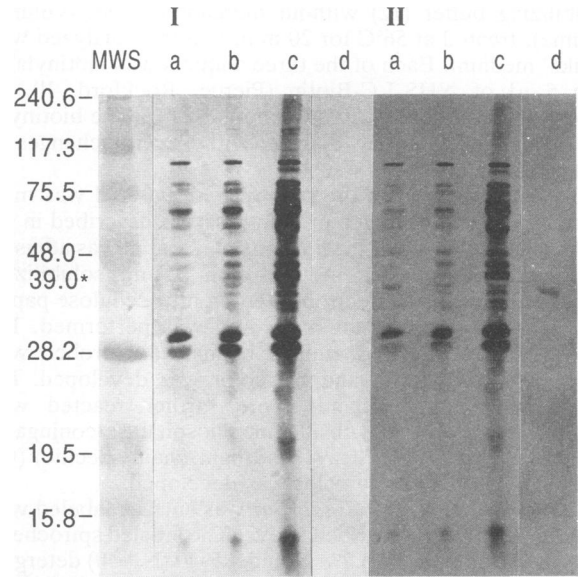

FIG. 4. Two-step blotting to determine the location of P39 within the spirochetes by use of biotinylated preparations of the spirochetes. (I) Step 1, reactivity with avidin-HRP. (II) Step 2, reactivity with NYSP39H. For each step, lanes a, b, c, and d represent, respectively, biotinylated whole spirochetes biotinylated and sonicated spirochetes, biotinylated and solubilized spirochetes, and nonbiotinylated control spirochetes. MWS, molecular weight standards, in thousands.

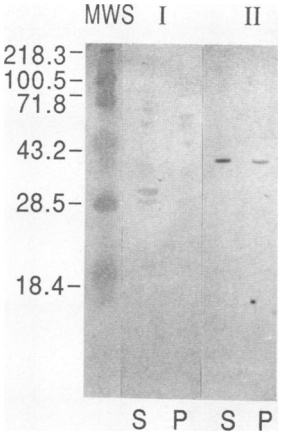

FIG. 5. Determination of the location of P39 within the spirochetes. (I) Immunoblotting with NYSP39H. (II) Immunoblotting with avidin-HRP. Lane S, supernatant containing NP-40-extracted material after centrifugation of biotinylated *B. burgdorferi*. Lane P, cell pellet. MWS, molecular weight standards, in thousands.

Furthermore, when membranes of the biotinylated spirochetes were extracted with NP-40, P39 did not react with the avidin-HRP probe, indicating that P39 is not accessible for surface biotinylation (Fig. 5). As a control, NYSP39H did react in the immunoblot test with the NP-40-extracted P39 band, indicating that P39 is a membrane component.

Immunoelectron microscopy showed that the polyclonal mouse anti-*B. burgdorferi* antibodies consistently labeled the cytoplasm, flagella, cytoplasmic membrane, and both the interior and the exterior surfaces of the cell wall (Fig. 6a and b). In contrast, NYSP39H essentially labeled only the cell region near the cytoplasmic membrane (Fig. 6c and e); on rare occasions, some cytoplasmic labeling was observed (Fig. 6d). No label was associated with the bacterial outer surface or flagella (Fig. 6e).

B. burgdorferi was detected in 9 of 14 ticks examined (64%) by the IFA test. The intensity of the fluorescence on the polylysine-fixed spirochetes was 2+ with NYSP39H.

DISCUSSION

Since they were first introduced, MAbs have played an increasingly important role in both the diagnostic and the basic aspects of medical sciences. They are used to localize and map antigens (15 and references cited therein) and to isolate (8, 23) variant organisms from bacterial cultures. These variants would not contain the antigen(s) which expresses the epitope, while the antigen-containing bacterium from the culture would die when reacted with the bactericidal MAb. Their application in antigenic diagnosis has improved the specificity of diagnostic tests for the detection of, e.g., hepatitis B surface antigen (28) and *B. burgdorferi* (4) in ticks and other insects (18).

Of all the techniques available for the specific detection of *B. burgdorferi* in ticks, e.g., PCR culturing, or dark-field microscopy, fluorescent-antibody microscopy is at present the most convenient tool. The use of MAbs to identify *B. burgdorferi* has been described elsewhere (15 and references cited therein). MAbs to various antigenic fractions, e.g., outer surface proteins (OspA and OspB) and flagellin, have been used to specifically detect *B. burgdorferi* in ticks. MAbs to flagellin may cross-react with spirochetes from other *Borrelia* species and with a number of gram-negative organisms making them unsuitable for the definitive identification of *B. burgdorferi*. The use of an MAb to one or the other major outer surface protein may be restricted because of the variation observed with antigens of various isolates of *B. burgdorferi*, to which that MAb may not react (1, 3, 16). We propose that MAb NYSP39H presents a significant advantage over MAbs that are specific for other antigenic fractions of *B. burgdorferi* and that have been used to identify *B. burgdorferi* in ticks.

NYSP39H reacted with 13 isolates of *B. burgdorferi*. P39 has not been detected in other spirochetes (24, 25, 26) of the same or other genera. It is also apparently conserved and has been detected in European and American isolates (26). However, Ma et al. (17) have reported that sera from patients with syphilis appear to react with P39. Whether these antibodies are directed to P39 or a comigrating band has yet to be demonstrated. Simpson et al. (26) have shown that the cloned 6.3-kb fragment that carries the gene encoding P39 also encodes a *B. burgdorferi*-specific 28-kDa antigen. NYSP39H does not appear to react with the 28-kDa antigen.

The localization of P39 antigen in *B. burgdorferi* was studied by biotinylation of the surface of whole spirochetes, by NP-40 extraction of the biotinylated membrane, and by immunoelectron microscopy. All three investigations showed that the P39 antigen is an integral part of the outer membrane but does not appear to be present on the surface of *B. burgdorferi*.

The infrequently encountered labeling found in the cytoplasm by immunoelectron microscopy may suggest a cytoplasmic origin for this antigen. Another explanation of the sparsity of the gold label is the sensitivity of P39 to the glutaraldehyde fixation procedure, resulting in a reduction in the antigenicity of P39. This reduction in antigenicity was also observed in the IFA test when the spirochetes were fixed in glutaraldehyde or other chemical fixatives. We also attempted to label the outer surface of the spirochetes by using a whole mount, negative-stain technique (13). While the polyclonal antibody labeled the surface, the control ascites fluid and the MAb to the 39-kDa antigen did not (results not shown).

B. burgdorferi was detected in 9 of 14 ticks examined (64%) by the IFA test. These findings were similar to the results of studies by Maupin et al. (20) and by Falco and Fish (11), who used adult ticks collected in Westchester County, N.Y. They showed infectivity rates of 49.7 and 55%, respectively. The relatively reduced fluorescence with the anti-P39 sera was not unexpected, since P39 is a minor antigenic component of *B. burgdorferi*. The advantage of NYSP39H over polyclonal anti-recombinant P39 rabbit sera is that the latter contains antibody to *E. coli*. In addition, the rabbit anti-*B. burgdorferi* antisera cross-reacted with all the spirochetes tested.

The epitope(s) recognized by both the monoclonal and the polyclonal antisera to P39 was apparently sensitive to the fixatives normally used in fluorescence microscopy, such as acetone, glutaraldehyde, and methanol. Therefore, either P39 was sensitive to these fixatives or the epitopes were made inaccessible to the MAb after acetone treatment. Kramer et al. (15) have ascribed the differences in staining with MAbs to OspA and OspB and MAbs to flagellin to alterations of the outer membrane of *B. burgdorferi* during preparation for immunofluorescence analysis. Polylysine was the only fixative that did not "destroy" the target epitope. In contrast, both the mouse and the rabbit antisera

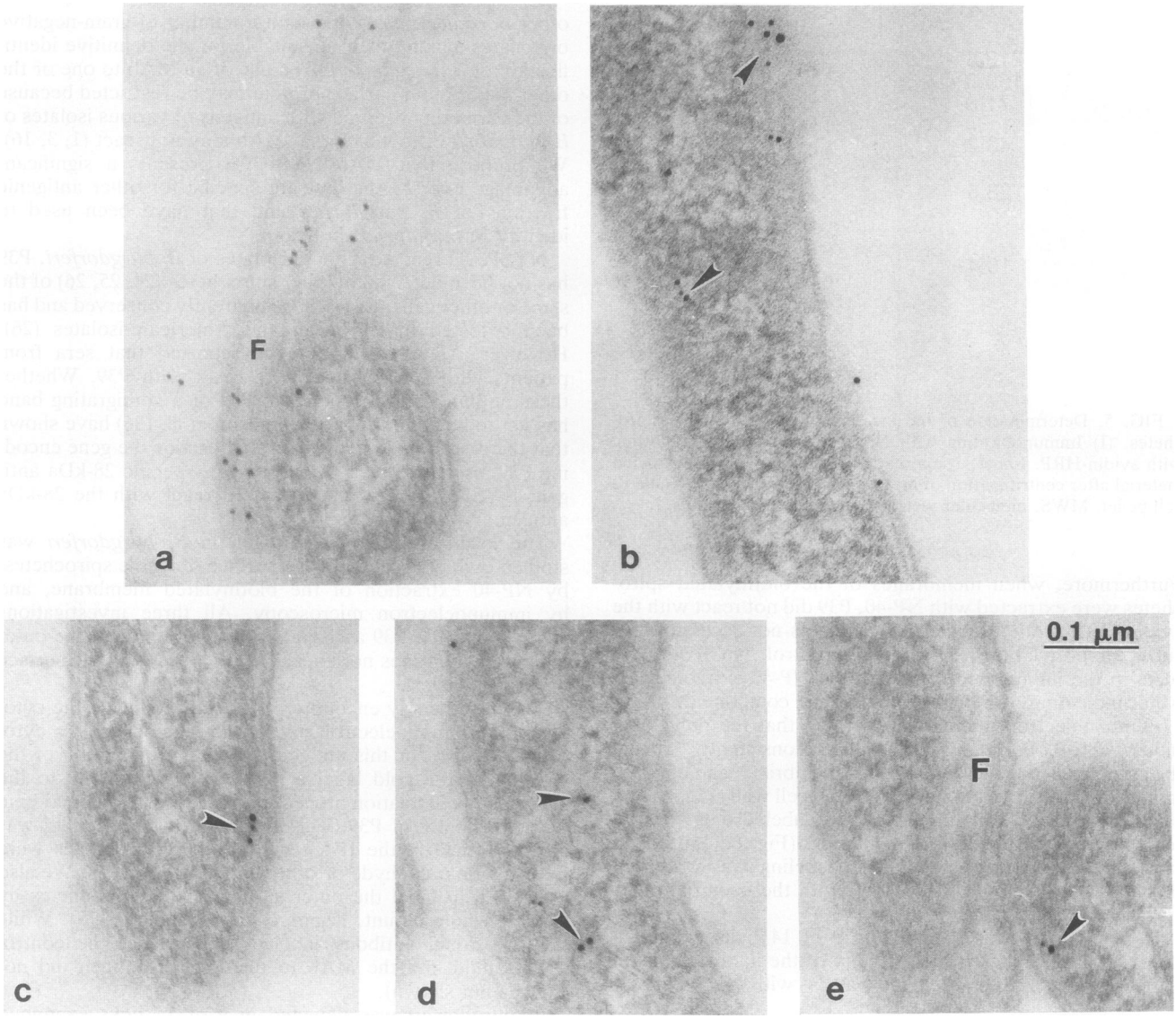

FIG. 6. Immunoelectron microscopy with colloidal gold markers to determine the location of P39 within the spirochetes. Polyclonal mouse antibodies to *B. burgdorferi* labeled flagella (F) as seen in panel a, and both internal and external regions of the cell (b, arrowheads). In contrast, MAb to P39 was found near the cytoplasmic membrane (c and e, arrowheads), sometimes within the cytoplasm (d, arrowheads), but never associated with flagella (f) (e).

to the whole spirochetes reacted equally (same titers) with the acetone- or polylysine-fixed spirochetes.

In conclusion, we have developed a MAb to P39 of *B. burgdorferi* which can be used alone or together with MAbs to the major surface proteins to detect *B. burgdorferi* in ticks and probably other vectors.

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