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Immune Capture and Detection of *Borrelia burgdorferi* Antigens in Urine, Blood, or Tissues from Infected Ticks, Mice, Dogs, and Humans

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Current biological and serological techniques for demonstrating infections by *Borrelia burgdorferi* can be inconclusive. In order to monitor Lyme borreliosis, we developed a rapid and sensitive assay for *B. burgdorferi* antigens in infected hosts. Polyclonal rabbit antisera were raised against membrane vesicles and an 83-kDa vesicle-associated protein band that was purified from in vitro *B. burgdorferi* cultures. Immunoglobulin G (IgG) antibodies were recovered from these sera and tested for a species-specific reaction with several geographically diverse *Borrelia* isolates by immunoblot analysis. Parlodion-coated electron microscope grids were activated with anti-vesicle $F(ab')_2$ fragments and then incubated with confirmed or experimental sources of spirochetal antigens. Such sources included cultured spirochetes; spirochete culture supernatants; samples of urine, blood, or serum from mice, dogs, and humans; triturates of *Ixodes* ticks; and bladder, spleen, liver, kidney, heart, or brain tissues from infected or control mice. Captured antigens were assayed by immune electron microscopy by using anti-83-kDa IgG antibodies and protein A-colloidal gold conjugates. The results indicated that *B. burgdorferi* appears to shed surface antigens which are readily detectable in urine, blood, and several organs from infected hosts. Such antigens were detectable in mouse urine at dilutions exceeding 10^{-6}. Intact spirochetes were frequently observed on grids incubated with blood, spleen, or bladder preparations, and *B. burgdorferi* was reisolated from the urinary bladders of all experimentally infected mice. These results indicated that *B. burgdorferi* antigens arise in a variety of host materials. Such antigens can be captured and identified with specific polyclonal antibodies, providing a sensitive assay for monitoring and studying Lyme borreliosis.

The immunological interactions between the Lyme disease spirochete, *Borrelia burgdorferi*, and its mammalian hosts are poorly understood (8–10, 12, 13, 16, 24, 29, 32–34). Although most mammalian hosts mount an antibody response to the spirochete, the antibodies are often serologically cross-reactive with other spirochetes (24, 25), and seronegative individuals with active infections have been encountered by standard screening criteria (10, 13, 16, 24, 32, 34). Furthermore, strain variation among *B. burgdorferi* isolates and antigenic variation within populations render immunodiagnostics based on monoclonal antibodies insensitive and unreliable for detection of circulating and excreted antigens in some hosts (2, 34). Therefore, clinical symptoms, patient history, and occasional primary isolations of the spirochete from blood or tissue biopsy specimens provide the bases for most diagnoses (6, 10, 12, 26). Such problems are often cited as factors influencing the reportedly poor diagnostic acumen for Lyme disease (13).

Considerable work is directed toward identifying conserved, species-specific cell surface antigens for diagnostic use and epidemiological and pathogenetic studies. Expression of outer surface protein A (OspA) is considered universal among *B. burgdorferi* isolates, but it is not present among related species (2, 4, 7, 16, 24, 34). Surface-exposed immunodeterminants on OspA appear to be antigenically variable, since no surface-reactive monoclonal antibodies to OspA have been reported to bind to all of the strains tested (2, 16, 34). Similarly, OspB is apparently unique to *B. burgdorferi*; however, the expression of *OspB* is reportedly variable in some strains (3, 28).

Recent experiments have shown that nuclease-protected DNAs are exported from *B. burgdorferi* cells in association with membrane vesicles (14). Indirect evidence suggests that these vesicles may be produced by spirochetes in vivo, providing a sustained antigenic challenge to hosts that maintain a limited population of spirochetes (13). To determine whether *B. burgdorferi* vesicles occur in experimentally infected mice, polyclonal rabbit sera were generated against vesicles and against a prominent, vesicle-associated protein band with an apparent mass of 83 kDa. Using these reagents, we developed an immune electron microscopic assay for first capturing and then identifying extracellular *B. burgdorferi* antigens. We then extended the study to include antigen detections in *Ixodes* ticks and in mouse, dog, and human samples. In this report, we describe the assay system and report the reliable and highly sensitive detection of antigens indicative of *B. burgdorferi* infections.

MATERIALS AND METHODS

Bacteria. The bacteria used in this study are described in Table 1. All *Borrelia* species were maintained in BSK II medium as described previously (1). *Leptospira interrogans* 23581, serotype icterohaemorrhagiae, was obtained from the American Type Culture Collection and was maintained in modified *Leptospira* medium as recommended by the American Type Culture Collection. Strain 23581 is an isolate from a human and is considered to be the type strain for *L. interrogans* (17). Whole cells and extracellular membrane

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TABLE 1. Bacteria used in this study[a]

Organism and strain	Source	Anti-MEP-GPA
Borrelia burgdorferi		
19678	*Peromyscus leucopus*, New York	+
20004	*Ixodes ricinus*, France	+
26816	*Microtus* species, Rhode Island	+
B31	*Ixodes dammini*, New York	+
G2	Human CSF, Germany	+
HB19	Human blood, Connecticut	+
Sh-2-82	*Ixodes dammini*, New York	+
Borrelia anserina	RML	−
Borrelia coriaceae CO53	*Ornithodorus coriaceus*, California	−
Borrelia hermsii HS1	*Onithodorus hermsi*, Washington	−
Borrelia parkeri	RML	−
Borrelia turicatae	RML	−
Leptospira interrogans	ATCC 23581	−

[a] Abbreviations: ATCC, American Type Culture Collection; GPA, protein A-colloidal gold conjugate; MEP, major extracellular protein; RML, Rocky Mountain Laboratory collection; CSF, cerebrospinal fluid.

vesicles were recovered by filtration and differential centrifugation as described previously (11, 14).

Antibodies. Supernatants from hybridomas 5332 (4) and 5TS (3) were used as sources of monoclonal antibodies directed against OspA and OspB, respectively. Polyclonal rabbit sera were raised against membrane vesicle concentrates and against the 83-kDa major extracellular protein band (MEP) that was electrophoretically purified from vesicles (11, 19). Emulsions of antigen and monophosphorylated lipid A and trehalose dimycolate (Ribi ImmunoChem, Inc., Hamilton, Mont.) were prepared according to the instructions of the manufacturer and were used as primary immunogens. Immunized rabbits were periodically boosted with antigen suspended in Dulbecco phosphate-buffered saline (dPBS pH 7.2). Sera were collected over a period of 10 weeks.

Immunoglobulin G (IgG) antibodies were purified from the sera by affinity chromatography with protein A-agarose (Sigma Chemical Co., St. Louis, Mo.). Eluted IgG was dialyzed overnight with water and lyophilized for storage.

For some experiments, $F(ab')_2$ fragments were produced from IgG directed against vesicles by passage through pepsin-agarose (Sigma) by using the buffers described previously (22). Cleaved IgG was subsequently passed through protein A-agarose; and the void volume was retained, dialyzed against water, and lyophilized. Both purified IgG antibodies and $F(ab')_2$ fragments were rehydrated at 1 mg/ml of dPBS prior to use.

Experimental mouse infections. White-footed mice (*Peromyscus leucopus*) from a captive colony which is free from infection with *B. burgdorferi* (28–30) were used as laboratory models of infection. These mice were experimentally infected with *B. burgdorferi* by intraperitoneal injection with 0.1 ml of passage 4 spirochetes suspended in dPBS at an optical density at 600 nm of 0.4 (approximately 10^7 bacteria) (28–30). The infected mice were sacrificed at 16, 39, and 69 days postinfection. Urine; blood; and urinary bladder, kidney, liver, spleen, heart, and brain tissues were collected from infected and uninfected animals. Infection was confirmed by culturing *B. burgdorferi* from triturated urinary bladders, as described previously (29, 30).

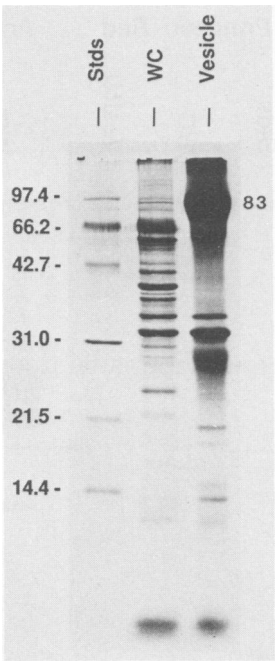

FIG. 1. Whole-cell (WC) and vesicle-associated proteins. Whole cells and purified vesicles were solubilized and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A 15% gel, which was stained with Coomassie brilliant blue, shows that vesicle preparations contain relatively few protein bands. These include bands that correspond by migration to OspA and OspB and a highly concentrated MEP with an apparent mass of 83 kDa. Vesicles appeared to lack detectable quantities of the 41-kDa endoflagellin band. Stds, molecular mass standards, which are indicated on the sides (in kilodaltons).

Human, canine, and tick materials. Human urine and blood samples, which were collected from patients with suspected Lyme borreliosis, were graciously provided by Paul Duray. The donors were chosen from among patients with histories of erythema migrans, arthritis, neurologic involvement, and/or congenital Lyme borreliosis (11a). Human urine and blood samples were also provided by laboratory volunteers. Although this study was not designed to examine the effectiveness of the antigen assay on standardized clinical human samples statistically, our results with human specimens were compared with serological data, patient histories, or both whenever possible. Urine and blood samples from a dog that was naturally infected in Bridgewater, N.J., were provided by Sara Stephens. The infected dog exhibited severe arthritic and neurological involvement, and a postmortem evaluation of tissue pathology and serology indicated acute Lyme borreliosis. *Ixodes dammini* ticks experimentally infected with *B. burgdorferi* JD1 were graciously provided by Joseph Piesman. Control *Ixodes pacificus* ticks were raised at Rocky Mountain Laboratories, and control canine urine was collected in Hamilton, Mont., an area that is nonendemic for Lyme disease.

Immunoblot analysis. Whole cells and vesicle preparations were solubilized and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis by using the discontinuous buffer system of Laemmli (21), with previously described modifications (18). Separated proteins were electroblotted onto nitrocellulose, blocked with 0.05% Tween 20, and probed with monoclonal or polyclonal IgG antibodies, as described previously (5). The resulting im-

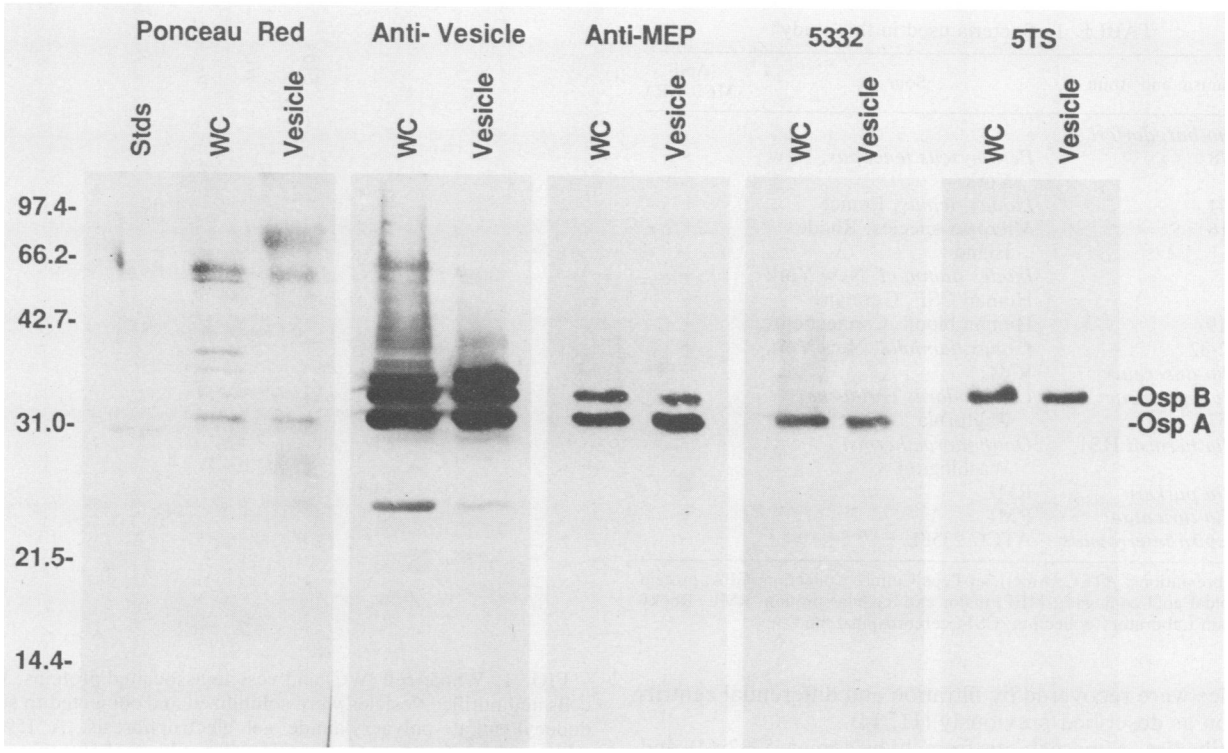

FIG. 2. Immunoblot analysis of antibodies used for precipitation and detection of *B. burgdorferi* antigens. Electroblotted whole-cell (WC) and vesicle proteins were stained with Ponceau red and probed with polyclonal rabbit IgG antibodies generated against extracellular vesicles, the 83-kDa MEP, or monoclonal antibodies 5332 or 5TS directed against OspA and OspB, respectively. Anti-vesicle IgG antibodies recognized several whole-cell and vesicle proteins. The anti-MEP IgG antibodies recognized proteins at 32 and 34 kDa in whole-cell and vesicle lanes corresponding to OspA and OspB. Stds, molecular mass standards, which are indicated on the left (in kilodaltons).

mune complexes were labeled with protein A-horseradish peroxidase and detected by a chromogenic assay.

Electron microscopy. Copper grids were coated with Parlodion and then incubated for 10 min at room temperature on a 6-μl droplet of anti-vesicle $F(ab')_2$ fragments dissolved at 1 mg/ml of dPBS. The grids were washed twice for 10 min with dPBS and then incubated for 10 min on a 6-μl droplet of antigen that was prepared as follows: urine, diluted 1:10, or as specified, in dPBS; blood, diluted 1:10 in dPBS; and ticks or organs, macerated in an equal volume of dPBS in glass tissue grinders and then diluted 1:10 in dPBS. After the grids were washed twice for 10 min each time on droplets of dPBS, they were incubated for 20 min at room temperature on 6-μl droplets of anti-MEP IgG antibodies dissolved at 50 μg/ml of dPBS. Two more dPBS washings were done, and then antigen-antibody complexes were labeled for 20 min with 6 μl of protein A-colloidal gold conjugates prepared by the methods of Robinson and coworkers (27). Two 5-min washings in dPBS were followed by two 5-min washes in 0.25 M ammonium acetate, and the grids were negatively stained with 0.5% ammonium molybdate (pH 6.5). The grids were dried in air and observed at 75 kV with a model HU-11E-1 transmission electron microscope (Hitachi, Ltd., Tokyo, Japan).

RESULTS

To determine the protein content of extracellular membrane vesicles of *B. burgdorferi*, whole cells and purified vesicles were solubilized and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 1). A 15% gel that was stained with Coomassie brilliant blue showed that relatively few protein bands that were present in whole-cell extracts were associated with vesicles. Vesicles contained two proteins which corresponded by migration to OspA and OspB and which reacted with monoclonal antibodies to OspA and OspB (see below). A highly concentrated MEP with an apparent mass of 83 kDa was resolved in vesicle extracts. No band with a similar mass was recovered from uninoculated medium by vesicle preparation methods or dehydration (data not shown). Additional proteins that appeared to be more concentrated in vesicles than in whole cells were resolved at 64, 30, 28, 21, 19, 15, and 14 kDa. Vesicle lanes lacked detectable quantities of the 41-kDa endoflagellin band.

To address whether membrane vesicles are released by *B. burgdorferi* in vivo, polyclonal rabbit antibodies were raised against vesicles and the 83-kDa MEP. After purification from collected sera, IgG antibodies from the rabbits were screened for reactivity with whole cells and vesicles by immunoblot analysis (Fig. 2). Blotted proteins were either stained with Ponceau red or probed with polyclonal IgG antibodies directed against extracellular vesicles or the MEP or with monoclonal antibodies to OspA and OspB, and then the proteins were labeled and detected as described above. Numerous whole-cell and vesicle proteins were recognized by anti-vesicle IgG antibodies. Polyclonal IgG antibodies from rabbits immunized with the MEP reacted primarily with proteins at 32 and 34 kDa, which, by electrophoretic migration, corresponded to OspA and OspB, respectively. Only minimal labeling of the 83-kDa immunogen occurred. The presence of OspA and OspB in whole cells and vesicles

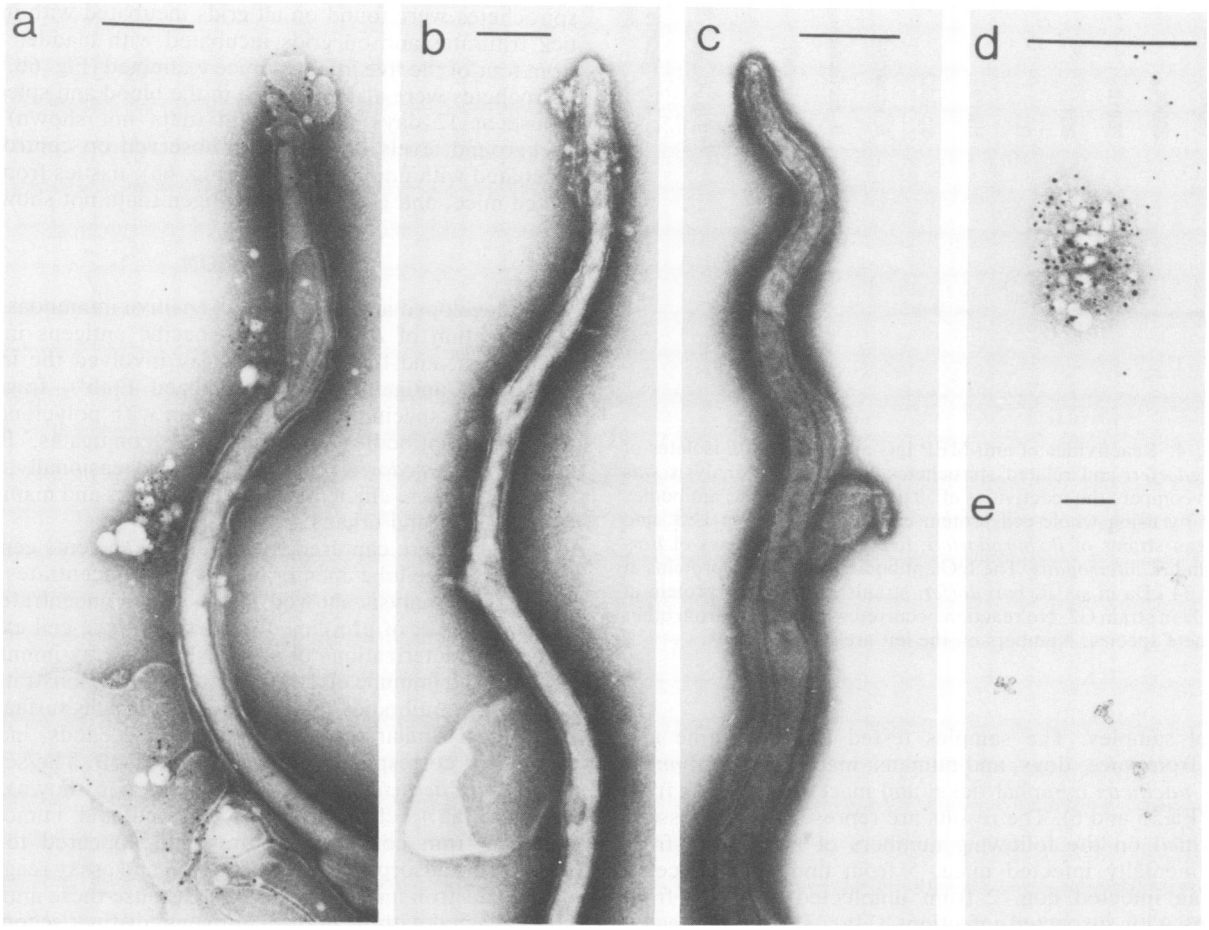

FIG. 3. Localization of epitopes recognized by IgG antibodies directed against the MEP. Parlodion-coated grids were primed with anti-vesicle $F(ab')_2$ fragments and then incubated with cultured *B. burgdorferi* cells (a and b), cultured *B. hermsii* (c), culture supernatant (d), or uninoculated culture medium (e). The grids were then probed with anti-MEP IgG antibodies (a, c, d, and e) or preimmune serum (b), labeled with protein A-colloidal gold conjugates, and examined by electron microscopy. Gold particles adhered to cell and vesicle surfaces and to flocculent material that surrounded the cells and that was present in culture supernatants. Only background levels of gold were observed on grids that were incubated with *B. hermsii* or medium or that were probed with preimmune serum. Bars, 200 nm.

was confirmed by reactivity with monoclonal antibodies 5332 and 5TS, respectively.

To determine whether the polyclonal anti-vesicle and anti-MEP antibodies could be used to concentrate and specifically label intact whole cells and elaborated vesicles, Parlodion-coated electron microscope grids were adsorbed with $F(ab')_2$ fragments made from anti-vesicle IgG antibodies. Such activated grids were incubated either with in vitro cultures of *B. burgdorferi* or *Borrelia hermsii* or with medium alone. Antigens that adhered to the grids were labeled with anti-MEP IgG antibodies and protein A-colloidal gold conjugates (Fig. 3). Spirochetes and vesicles from *B. burgdorferi* and *B. hermsii* cultures adhered to the activated grids. Heavy labeling was evident on the surfaces of *B. burgdorferi* cells and vesicles and on the flocculent material surrounding these structures (Fig. 3a). Structurally similar material with heavy label was evident in *B. burgdorferi* culture supernatants (Fig. 3d). Sparse deposition of gold on the control grids containing cultured *B. hermsii* (Fig. 3c), uninoculated medium (Fig. 3e), or *B. burgdorferi* incubated with preimmune serum (Fig. 3b) was considered nonspecific background labeling.

To determine the specificity of the anti-MEP polyclonal IgG antibodies, we assayed seven strains of *B. burgdorferi*, five other *Borrelia* species, and the distantly related spirochete *L. interrogans* by immune electron microscopy (Table 1) and by immunoblot analysis (Fig. 4). By microscopy, all seven *B. burgdorferi* isolates from the United States and Europe were specifically labeled, whereas *L. interrogans* and five additional *Borrelia* species, including *B. hermsii*, retained only background levels of gold. Similarly, immunoblots showed that the anti-MEP IgG antibodies reacted with proteins ranging from 32 to 35 kDa in each strain of *B. burgdorferi*, but they did not react with proteins from equivalent whole-cell extracts from other *Borrelia* species or *L. interrogans* (Fig. 4). A single band at 32.5 kDa was labeled by the IgG antibodies in the G2 strain of *B. burgdorferi*. Counter stains of such blots showed that strain G2 did not contain predominant bands that corresponded by electrophoretic migration to OspA and OspB in the other *B. burgdorferi* strains. Rather, the band that reacted with the anti-MEP IgG antibodies was the only major protein observed between 30 and 40 kDa (data not shown).

Activated grids were then incubated with possible in vivo sources of *B. burgdorferi* antigens to assess the possibility of applying this antigenic capture and detection system to

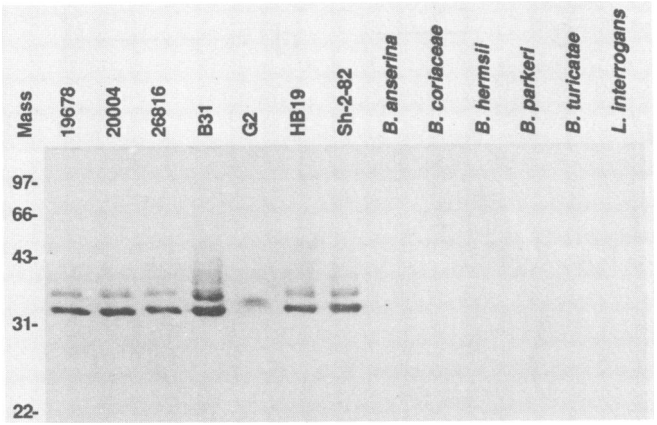

FIG. 4. Reactivities of anti-MEP IgG antibodies with isolates of *B. burgdorferi* and related spirochetes. Immunoblot analysis was used to compare the reactivities of 50 μg of anti-MEP IgG antibodies per ml by using whole-cell protein extracts from seven U.S. and European strains of *B. burgdorferi*, five additional species of *Borrelia*, and *L. interrogans*. The IgG antibodies recognized proteins at 32 and 34 kDa in six *B. burgdorferi* strains and a single protein at 32.5 kDa in strain G2. No reaction occurred with proteins from other spirochete species. Numbers on the left are in kilodaltons.

clinical samples. The samples tested included urine and blood from mice, dogs, and humans; macerated *I. dammini* and *I. pacificus* nymphal ticks; and macerated tissues from mice (Fig. 5 and 6). The results are representative of assays performed on the following numbers of samples: 5 from experimentally infected mice, 5 from uninfected mice, 1 from an infected dog, 2 from uninfected dogs, 39 from humans with suspected infections (11a), 3 from uninfected humans, 6 from experimentally infected ticks, and 3 from uninfected ticks. For each fluid or tissue sample from infected hosts, equivalent samples from uninfected animals were examined. Because grids incubated with such samples retained little observable material, photomicrographs of most controls are not shown.

Capture and labeling of aggregated flocculent antigens were evident in the urine and blood of all infected mice examined (Fig. 5a and b). Some fields contained labeled membranous structures resembling vesicles (Fig. 5a, inset). We observed similar flocculent material in urine from a dog (Fig. 5c) and a human (Fig. 5d) with clinically confirmed Lyme borreliosis (see Materials and Methods). Gold-labeled antigen aggregates could still be observed after a 10^6-fold dilution of urine from an infected mouse. No evidence of material specifically labeled with gold was detected in samples from uninfected laboratory mice or humans (Fig. 5e and f) or from dogs living in nonendemic Hamilton, Mont. (data not shown). Of the 39 urine samples from suspected human cases (see Materials and Methods), 1 urine sample was negative, whereas 38 samples had aggregates of flocculent antigen.

The macerated tissues that we examined included whole, laboratory-raised *I. dammini* and *I. pacificus* nymphal ticks and urinary bladders, spleens, livers, hearts, brains, and kidneys from mice (Fig. 6a to g, respectively). With the exception of kidney specimens, each tissue from infected animals contained aggregates of flocculent antigen. Although kidney tissues from infected mice were seen to be labeled more densely than kidney tissues from uninfected control animals were, antigen aggregates were not resolved. Intact spirochetes were found on all grids incubated with infected tick triturates and on grids incubated with bladder tissues from four of the five infected mice examined (Fig. 6b, inset). Spirochetes were also observed in the blood and spleen of a mouse at 12 days postinfection (data not shown). Only background levels of gold were observed on control grids incubated with uninfected ticks (Fig. 6h), tissues from uninfected mice, and grids lacking antigen (data not shown).

DISCUSSION

We developed and evaluated a sensitive immunoassay for the detection of *B. burgdorferi*-specific antigens in ticks, mice, dogs, and humans. The assay involved the immune capture of antigens with immobilized $F(ab')_2$ fragments, followed by specific antigen detection with polyclonal anti-MEP IgG antibodies and protein A conjugates. By this assay, *B. burgdorferi* antigens and, occasionally, intact spirochetes were efficiently detected in ticks and mammalian urine, blood, and organs.

Antigens were captured with $F(ab')_2$ fragments generated against extracellular membrane vesicle concentrates. Electrophoretic analysis showed that vesicle concentrates contained a subset of proteins observed in whole-cell extracts. Initial characterizations of anti-vesicle sera by immunoblot analysis and immune electron microscopy demonstrated that serum IgG antibodies labeled cell and vesicle surfaces and recognized similar whole-cell and vesicle bands, including bands that corresponded to OspA and OspB. The sera also labeled *B. hermsii* cell surfaces (data not shown). Anti-vesicle $F(ab')_2$ effectively concentrated and immobilized antigens from complex mixtures and appeared to block nonspecific adsorption of host material and assay reagents to coated electron microscopic grids. Because these antibodies cross-reacted with *B. hermsii* antigens, distinct second antibodies that had no apparent intrageneric cross-reactivity were used to detect antigens specific to *B. burgdorferi*.

The detection antibodies were generated against the 83-kDa MEP, which was the most prominent band in gels containing vesicle-associated proteins. Surprisingly, immunoblots showed that the resulting polyclonal sera recognized proteins at 32 and 34 kDa that corresponded to OspA and OspB, respectively, but had relatively weak activity with the original immunogen. Subsequent studies have demonstrated that the 83-kDa band may represent a surface-exposed complex of multiple vesicle-associated proteins, which includes OspA and OspB (10a). For example, treatment of both intact vesicles and excised MEP with certain endo- and exoglycosidases results in simultaneous decreases in the apparent quantity of the MEP and increases in both the staining of OspA and OspB and the labeling of these proteins with monoclonal antibodies (10a). Whether extensive glycosylation of proteins migrating at 83 kDa or other factors obscure this protein's major immunodeterminants on electroblots is unclear. Further molecular analyses of this protein band should help to define the relationships between MEP, OspA, and OspB and determine whether an 83-kDa *B. burgdorferi* protein, which was recently cloned and expressed by LeFebvre and coworkers (23), constitutes or is distinct from MEP.

As with previous characterizations of monoclonal antibodies directed against OspA and OspB (2–4, 16, 28, 34), the anti-MEP polyclonal IgG antibodies were species specific. Furthermore, unlike such monoclonal antibodies that invariably fail to bind to some strains (3, 28, 34), this polyclonal antibody bound to the cell surfaces and to electroblotted

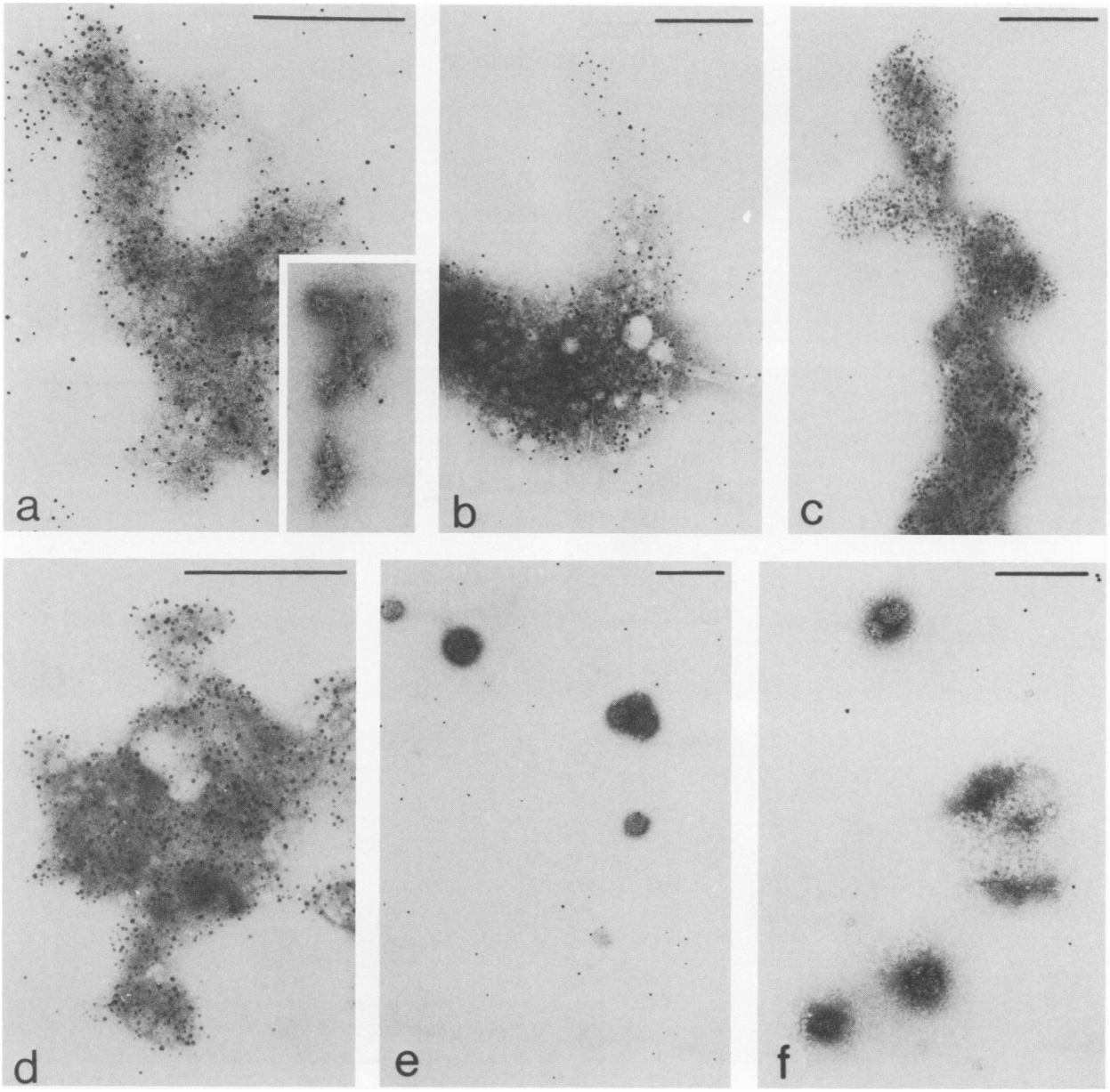

FIG. 5. Immune electron microscopic detection of *B. burgdorferi* antigens in mammalian urine and blood. Parlodion-coated grids were adsorbed with anti-vesicle $F(ab')_2$ fragments, incubated with urine or blood samples, probed with anti-MEP IgG antibodies, and labeled with protein A-colloidal gold conjugates. Heavily labeled flocculent antigens were aggregated on grids incubated with urine from an experimentally infected mouse, a naturally infected dog, and a naturally infected human (a, c, and d, respectively). Similar material was detected in infected mouse blood (b). Labeled membrane vesicles were occasionally observed (a, inset). No specific labeling was observed in urine and blood from uninfected mice (e and f). Bars, 200 nm.

proteins from all strains of *B. burgdorferi* tested, including geographically diverse isolates. We presume that the polyclonal IgG antibodies bind to multiple epitopes on recognized proteins. Hence, divergence among *B. burgdorferi* strains, reflected by amino acid sequence variation within these proteins, could occur without the complete loss of recognition by anti-MEP IgG antibodies.

When these reagents were used to examine experimentally infected mice by electron microscopy, we detected aggregates of *B. burgdorferi* antigens in urine, blood, and macerated urinary bladder, spleen, liver, and brain tissues. Although, aggregated antigens were not observed in kidney tissue, the relatively dense deposition of gold in kidney tissue preparations suggested that *B. burgdorferi* antigens were present. The possibility that the antigens fail to aggregate or that aggregated antigens dissociate under the unique physiological conditions of pH or salt found in kidneys has not been pursued. Minimal background labeling on control grids lacking antigen and grids incubated with material from uninfected mice and humans indicated that gold deposition in this assay was specific for *B. burgdorferi* antigens.

Intact spirochetes were observed on grids incubated with urinary bladder tissues from four of five mice from which such tissues were obtained. Spirochetes were also detected

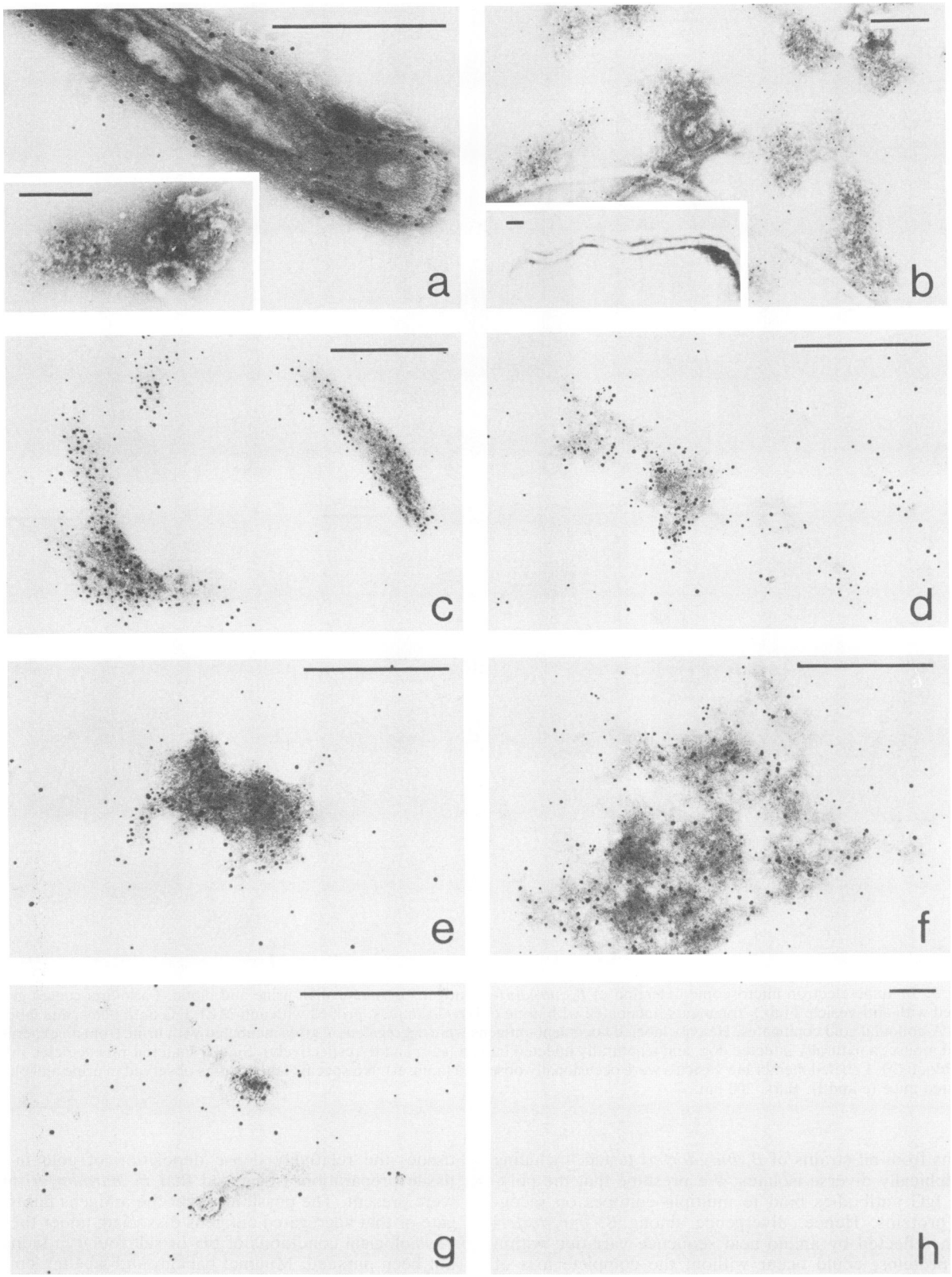

FIG. 6. Detection of *B. burgdorferi* antigens in macerated ixodid ticks and mouse tissues. Electron microscope grids were activated as described in the text and incubated with macerated tissue. Precipitated antigens were labeled with anti-MEP IgG antibodies and protein A-colloidal gold conjugates. When examined, flocculent antigens were observed on grids incubated with infected ticks and urinary bladder, spleen, liver, heart, and brain tissues from infected *P. leucopus* (a through f, respectively). Grids with infected *P. leucopus* kidney tissue had relatively dense labeling, but antigen aggregates were not observed on such grids (g). Little gold was observed on control grids incubated with uninfected ticks (h), equivalent tissues from uninfected mice, and grids lacking antigen (data not shown). Bars, 200 nm.

in all infected ticks, blood from a single mouse, and spleen samples from mice. Furthermore, a spirochete was observed in 1 μl of human urine (data not shown), suggesting that this detection system may facilitate studies of tissue involvement currently complicated by difficulty in demonstrating spirochetes in infected hosts (10, 12, 30).

Preincubation of the grids with anti-vesicle $F(ab')_2$ fragments dramatically increased the sensitivity of antigen detection, particularly in complex samples such as blood and macerated tissues (data not shown). Flocculent antigen was detectable in these samples without preincubation, but the grids also contained considerable quantities of unlabeled host material. Control experiments showed that once the grids were activated with $F(ab')_2$ fragments, further adsorption by material from uninfected animals and by proteins such as protein A and IgG from blood samples or purified preparations was negligible. Apparently, the $F(ab')_2$ fragments functioned both by capturing *Borrelia* antigens and by blocking nonspecific adsorption of eukaryotic material.

Vesicles were resolved on the surfaces of spirochetes recovered from infected ticks and mouse tissues, indicating that these vesicles are formed by *B. burgdorferi* in vivo. Gold-labeled, membranous vesicles were also observed in urine and blood. The majority of specific gold labeling occurred on flocculent material detected in *B. burgdorferi* cultures and in all infected mammals examined. While structurally similar material was frequently observed on and surrounding cell surfaces, its exact nature is unknown. Negative stains showed that the material resembled surface layers, or s layers, reported for *B. burgdorferi* (20) and other spirochetes (31). Since monoclonal antibodies and polyclonal IgG antibodies that recognize OspA and OspB bind this material (10a) and since previous work showed that *B. burgdorferi* sloughs OspA from cell surfaces (4), OspA and OspB may occur within an s layer.

The detection of *B. burgdorferi* DNA and antigens in human urine has been reported recently (15, 16). This study confirmed the presence of *B. burgdorferi* antigens in urine and provides methods that may enhance the reported sensitivity of detection (16). This study also showed that extracellular *B. burgdorferi* antigens occur in tissues in which spirochetes are infrequently reported (12). Such results suggest either that large quantities of circulating antigen are deposited in these tissues or that antigens are secreted by a limited number of meandering spirochetes and that the antigens persist in situ. The possible pathological effects of this material are unknown. Determination of the nature of the antigens and the mechanisms behind their deposition may lead to a better understanding of the pathogenesis of Lyme disease. The reagents developed in this study should facilitate such determinations.

The combination of immune capture with polyclonal antigen detection should both reduce false-negative assays for *B. burgdorferi* that can result from minor antigenic variation and enable reliable demonstrations of this spirochete and its products in vectors, reservoir hosts, domestic animals, and humans. Although this capture and detection system effectively resolved *B. burgdorferi* antigens under controlled laboratory conditions, the possibility that the system could prove to be statistically valuable for medical or veterinary diagnostics remains to be determined. Yet, this system, which was designed for careful electron microscopic examination of captured antigens, should also be readily adaptable to clinical immunodiagnostic protocols such as microtiter enzyme-linked immunosorbent assays.

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