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REACTIVITY OF WHITE-FOOTED MOUSE AND RACCOON SERA TO WHOLE CELL AND RECOMBINANT ANTIGENS OF *BORRELIA BURGDORFERI*

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ABSTRACT: Serum samples were collected from white-footed mice (*Peromyscus leucopus*) and raccoons (*Procyon lotor*) during 1983, 1984, and 1990 through 1993 in Connecticut (USA) and were tested in enzyme-linked immunosorbent assays (ELISA) against whole cell *Borrelia burgdorferi* sensu stricto (strain 2591) and the following recombinant antigens of this spirochete: p41-G (an immunogenic epitope of flagellin), outer surface protein (OSP)A, and OSPB. Antibodies were most frequently detected when whole cell antigen was used in the analyses. Reactivity to highly specific recombinant antigens also occurred and was particularly helpful in verifying *B. burgdorferi* infection. Geometric mean antibody titers for assays with whole cell antigen ranged from 453 to 2, 363 and were at least two-fold higher than geometric means calculated for tests with recombinant antigens, which ranged from 226 to 640. With greater sensitivity, an ELISA with whole cell antigen is preferred for determining presence of antibody in sites enzootic for Lyme borreliosis. However, use of highly specific recombinant antigens, particularly OSPA and OSPB, in an ELISA can provide supportive information in ecological studies of this disease.

Key words: *Borrelia burgdorferi*, Lyme borreliosis, antibodies, enzyme-linked immunosorbent assay, white-footed mouse *Peromyscus leucopus*, raccoon *Procyon lotor*.

INTRODUCTION

Human cases of Lyme borreliosis have been reported in numerous areas of the United States and Eurasia (Sigal, 1988; Steere, 1989; Tsai et al., 1989). It often is assumed that persons are exposed to ticks and acquire *Borrelia burgdorferi* sensu lato infections close to their homes or on trips to tick-infested recreational areas. In most instances, however, the actual sites for infection are unknown. To identify foci, *B. burgdorferi* can be isolated from the tissues of captured rodents or their ticks (Anderson et al., 1985; Anderson and Magnarelli, 1992). Such direct evidence of this spirochete's presence in white-footed mice (*Peromyscus leucopus*), an important reservoir for this spirochete in nature (Levine et al., 1985), or in other vertebrate or tick hosts has been used to verify numerous foci for Lyme borreliosis in North America and Europe (Loken et al., 1985; Anderson, 1989). Although culturing provides more definitive results than antibody detection assays, assays have been used to provide supportive epizootiological information (Godsey et al., 1987; Magnarelli et al., 1990). Whole cells of *B. burgdorferi* have been used almost exclusively in enzyme-linked immunosorbent assays (ELISA) to detect antibodies to *B. burgdorferi*. Subunit and recombinant antigens have been tested to improve sensitivity and specificity in laboratory diagnosis of human infections (Hansen et al., 1988; Magnarelli et al., 1989). Our objective was to screen highly specific recombinant antigens of *B. burgdorferi* in an ELISA and to compare these results with those of an ELISA containing whole cell antigen for serum antibody detection in white-footed mice and raccoons (*Procyon lotor*), animals frequently parasitized by *Ixodes scapularis*.

MATERIALS AND METHODS

Blood samples were obtained from white-footed mice primarily between April and Sep-

tember of 1983, 1984, and 1990 through 1993. Animals were captured in Sherman box traps (H.B. Sherman Traps, Inc., Tallahassee, Florida, USA) in Avon, Barkhamsted, Chester, Deep River, East Haddam, Gaylordsville, Haddam, Lakeville, Lyme, New Milford, Newtown, Old Lyme, West Hartford, and Stamford, Connecticut (USA). *Ixodes scapularis*, the main tick vector of *B. burgdorferi* sensu stricto in northeastern and upper midwestern United States, is abundant in Chester, Deep River, East Haddam, Lyme, Old Lyme, and Stamford, but is rarely encountered in the remaining towns. Towns located at the periphery of the sampling region are Stamford in southwestern Connecticut (41°5′30″N, 73°32′30″W), Lakeville in the northwestern part of the state (41°57′45″N, 73°26′00″W), and Old Lyme in eastern Connecticut (41°20′30″N, 72°17′30″W).

We captured 48 raccoons during the summer in Tomahawk traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) in East Haddam, Guilford, Lyme, Newtown, and Woodbridge, Connecticut during 1978 through 1983. These towns are located within the geographic range of sampling areas included for the collection of white-footed mice. *Ixodes scapularis* rarely was collected in Newtown during this sampling period. All serum specimens were stored at −60 C until analyses.

Whole cells of *B. burgdorferi* sensu stricto (strain 2591) were obtained from cultured stock (Magnarelli et al., 1984). This strain has been subcultured hundreds of times, and washed whole cells have been used extensively in antibody tests at The Connecticut Agricultural Experiment Station, New Haven, Connecticut. Recombinant antigens of *B. burgdorferi* were prepared at Yale University, New Haven, by Fikrig et al. (1992) and included outer surface protein (OSP)A, OSPB, and an immunogenic epitope of flagellin (p41-G). Of these, OSPA is most specific to *B. burgdorferi*. Based on size and reactivity with monoclonal antibodies, OSPB is more heterogeneous than OSPA (Barbour et al., 1984). Either or both of these proteins are recognized more frequently immunologically by human beings during later stages of Lyme borreliosis than during early weeks of infection (Wilske et al., 1988; Dressler et al., 1993). Previous use of p41-G as antigen in an ELISA (Magnarelli et al., 1992a) improved the specificity of human antibody tests, but shared antigenicity with treponemes still was evident. All recombinant antigens were expressed and purified as fusion proteins in *Escherichia coli* with glutathione transferase. Prior to use in ELISA, a commercially available assay (Bio-Rad, Richmond, California, USA) was used to determine protein concentrations of stock antigens to facilitate standardization. Bovine serum albumin (2 mg per ml) was purchased (Pierce, Rockford, Illinois, USA) and used as a standard in this test.

Details on materials and methods, including the sources of most control sera, used in analyses for serum antibodies by an ELISA with whole cell *B. burgdorferi* have been reported for mice (Magnarelli et al., 1992b) and raccoons (Magnarelli et al., 1991). Checkerboard titrations were used to determine optimal working concentrations of diluted antigens and positive sera. Whole cell and recombinant antigens were standardized to 3 μg and 5 μg of protein per ml, respectively. In analyses of mice, cutoff values for positive results were calculated by testing 20 negative sera obtained from white-footed mice collected in Avon, Barkhamsted, Gaylordsville, Lakeville, New Milford, Newtown and West Hartford, towns where *I. scapularis* rarely is encountered and from five white-footed mice born in the laboratory. The laboratory-bred animals had no *B. burgdorferi* infections, as determined by attempts to culture the bacterium from bladder, kidney, and spleen tissues (Magnarelli et al., 1994). There were no differences in the reactivity of sera from these two groups in an ELISA. A net optical density (OD) value of 0.04 was considered positive for all serum dilutions ≥1:160 when p41-G was used as antigen. Optical density values of 0.05 and 0.08 were positive in assays with OSPA and OSPB for serum dilutions of 1:160, while an OD value of 0.04 was evidence for antibody presence in serum dilutions ≥1:320. Similar critical regions were computed in analyses of raccoon sera with recombinent antigens. We tested 25 negative sera from animals captured in western Connecticut from non-enzootic areas for Lyme borreliosis. Optical density values of 0.23, 0.12, and 0.06 were positive in an ELISA with p41-G antigen for serum dilutions of 1:160, 1:320, and ≥1:640, respectively. For assays with OSPA or OSPB coated to the plates, OD values of 0.08 and 0.13 were positive for a serum dilution of 1:160, respectively. At higher serum dilutions of ≥1:320, OD values of 0.04 and 0.08 were evidence for antibodies to OSPA and OSPB, respectively. Critical regions for positive results were established by statistical analyses of OD readings for each serum dilution of negative samples as described by Magnarelli et al. (1984). Tests included positive and negative control sera, steps to ensure standardization of reagents and to monitor variability of nonspecific background readings, and controls to check for false positive reactions due to the presence of glutathione transferase when recombinant antigens were used. Antigen reactivity in ELISA was further verified by testing murine monoclonal antibodies (H5332, H6831, and H9724) that contained

TABLE 1. Reactivity of white-footed mouse and raccoon sera to whole cells and recombinant antigens of *Borrelia burgdorferi* in an ELISA, Connecticut, 1983 to 1993.

Hosts	Potential for exposure to *B. burgdorferi*	Total serum specimens tested	Number (%) of serum specimens positive to			
			Whole cells	p41-G	OSPA	OSPB
Mice	high[a]	36	26 (72)	16 (44)	12 (33)	7 (19)
	low[b]	23	0	3 (13)	4 (17)	2 (9)
Raccoons	high[c]	14	11 (79)	3 (21)	4 (29)	2 (14)
	low[d]	34	4 (12)	1 (3)	0	4 (12)

[a] Chester, Deep River, East Haddam, Haddam, Lyme, Old Lyme, and Stamford.
[b] Avon, Barkhamsted, Gaylordsville, Lakeville, New Milford, Newtown, and West Hartford.
[c] East Haddam, Guilford, and Lyme.
[d] Newtown and Woodbridge.

antibodies to OSPA, OSPB, and flagellin of *B. burgdorferi*.

RESULTS

Mouse and raccoon sera were positive in an ELISA containing whole cell or recombinant antigens; percent positivity usually was highest in assays with whole cell antigen (Table 1). In areas considered to be highly enzootic for Lyme borreliosis, seropositivity was likewise relatively high for mouse sera (44%) and raccoon sera (21%) analyzed with the p41-G antigen. When comparing results of tests with OSPA and OSPB antigens, animal sera from highly enzootic foci more frequently were positive when plates contained OSPA. For samples collected in areas with little or no evidence for *B. burgdorferi*, no mouse sera were positive to whole cell antigen. Antibodies, however, were detected to p41-G, OSPA, or OSPB antigens in four or fewer serum specimens of the 23 tested. Similar results were recorded when raccoon sera were tested from towns where *I. scapularis* and Lyme borreliosis were rare. Four or fewer serum samples were positive in ELISA with whole cell, p41-G or OSPB antigen of the 34 sera tested. There was no reactivity in plates coated with OSPA antigen.

Antibody titers to whole cell *B. burgdorferi* usually were higher than those recorded when recombinant antigens were used (Table 2). For mouse and raccoon sera collected from highly enzootic areas and tested with whole cell antigen, the highest geometric mean titer was 2,363. In contrast, a maximal titer of 453 was recorded when recombinant antigens were used. Similarly, maximal antibody titers of 1:20,480 and 1:5,120 were recorded when whole cell antigen was tested with mouse and raccoon sera, respectively.

The variable pattern of serum reactivity to whole cell and recombinant antigens was evident for both animal species. Most serum samples positive in assays with whole cell *B. burgdorferi* were reactive to one or more recombinant antigens (Table 3). There were instances, however, when 13 mouse and eight raccoon sera were positive in tests with p41-G, OSPA, or OSPB without showing reactivity to whole cell antigen. At serum dilutions of $\geq$1:160, 16 mouse sera contained antibodies to OSPA, while nine sera were positive in ELISA with OSPB. Of the 23 raccoon sera positive to whole cell or recombinant antigens, 12 had antibodies to whole cell antigens. Antibody presence in assays with OSPA or OSPB was noted but was less frequent than reactivity to whole cell antigen.

DISCUSSION

Mouse and raccoon sera, which contained antibodies to whole cell *B. burgdorferi*, also were positive to recombinant antigens. As in previous work with human, dog, equid, and cottontail rabbit sera (Ber-

TABLE 2. Antibody titers for positive white-footed mouse and raccoon sera tested in an ELISA with whole cell or recombinant antigens of *Borrelia burgdorferi*, Connecticut, 1983 to 1993.

Site endemicity	Antigens	Antibody titers: Mice: Total positive sera	Mice: Ranges[a]	Mice: Geometric means	Raccoons: Total positive sera	Raccoons: Ranges[a]	Raccoons: Geometric means
High	Whole cell	26	160–20,480	2,363	11	160–5,120	1,128
	p41-G	16	160–640	247	3	160–640	403
	OSPA	12	160–640	254	4	320–640	453
	OSPB	7	160–640	390	2	160–320	226
Low	Whole cell	0	—	—	4	160–640	453
	p41-G	3	160–320	320	1	320	—
	OSPA	4	160–640	269	0	—	—
	OSPB	2	160–320	226	4	320–2,560	640

[a] Reciprocal antibody titers.

land et al., 1991; Collins and Peltz, 1991; Magnarelli et al., 1992a; Fikrig et al., 1993), recombinant antigens can be used in an ELISA to obtain more specific information on immunologic responses by mice and raccoons. Reactivity to OSPA and OSPB was particularly helpful because it demonstrated a more specific response to *Borrelia* spp. spirochetes. Therefore, the use of purified, highly specific OSPA antigen to detect serum antibodies can help verify *B. burgdorferi* presence in suspected foci. However, assays with recombinant antigens were less sensitive than an ELISA with whole cell *B. burgdorferi*. Thus, multiple antigens of this bacterium are immunologically recognized by mice and raccoons but some polypeptides, such as OSPA, are not always recognized. Determining prevalence of seropositive specimens at foci probably would be more accurate if whole cell antigen was used in assays. Further studies, including immunoblotting techniques, should be conducted to identify polypeptides of *B. burgdorferi* that most frequently are recognized by humoral response. Although purified preparations of P39 and OSPC were unavailable when the present study was conducted, particular attention should be given to these antigens. Both polypeptides are recognized during the early stage of human Lyme borreliosis infections (Simpson et al., 1990; Wilske et al., 1988). A mixture of highly specific recombinant antigens in an ELISA may improve sensitivity.

In some instances, there was reactivity to recombinant antigens, but results of an ELISA with whole cell *B. burgdorferi* were negative. Whole cell sonicates of this spi-

TABLE 3. Summary of positive serologic test results in an ELISA with whole cell or recombinant antigens of *Borrelia burgdorferi*, Connecticut, 1978 to 1983.

Antigens tested[a]	Total number of positive serum samples: White-footed mice	Raccoons
Whole cells only	4	12
Whole cells and p41-G	11	0
Whole cells, p41-G, and OSPA	2	0
Whole cells, p41-G, OSPA and OSPB	1	0
Whole cells and OSPA	5	1
Whole cells, OSPA, and OSPB	1	1
Whole cells, P41-G, and OSPB	2	0
Whole cells and OSPB	0	1
p41-G only	3	2
p41-G and OSPA	0	2
p41-G, OSPA, and OSPB	0	0
p41-G and OSPB	0	0
OSPA only	5	0
OSPA and OSPB	2	0
OSPB only	3	4

[a] Antigens analyzed separately with sera.

rochete contain numerous proteins. When coated to immunoplates, some spirochetal proteins may block or interfere with antibody reactivity to other key immunodominant polypeptides. Therefore, to optimize antibody-antigen reactivity in an ELISA, it is especially important to include the most important antigens. Immune responses vary among animal species, as observed in the present study, and can even differ among individuals of a given species. Variable immune responses among persons who had Lyme borreliosis are well documented (Dressler et al., 1993). Therefore, these factors should be considered in efforts to improve diagnostic tests for any species. Moreover, serum reactivity to OSPA or OSPB without detectable antibodies to whole cell antigen may be epizootiologically significant. Even though some sites were considered to be of low endemicity for Lyme borreliosis, as determined by reports of human infections, our serologic test results provide evidence for mouse and raccoon exposure to *B. burgdorferi*. Culturing this bacterium from these or other mammalian species is ultimately needed to verify infection.

Serologic tests can provide clues as to which vertebrate hosts have had exposure to *B. burgdorferi*. The role of white-footed mice as reservoirs for this spirochete in nature is clear (Donahue et al., 1987). This rodent has been the focus of numerous field studies where tick parasitism, isolation of *B. burgdorferi*, and efforts to suppress Lyme borreliosis have been considered. For this reason, greater attention should be given to improve antibody or antigen detection assays for this species.

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