JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1997, p. 169–173 Vol. 35, No. 1
0095-1137/97/$04.00+0

Serologic Diagnosis of Canine and Equine Borreliosis: Use of Recombinant Antigens in Enzyme-Linked Immunosorbent Assays

LOUIS A. MAGNARELLI,[1]* RICHARD A. FLAVELL,[2] STEVEN J. PADULA,[3] JOHN F. ANDERSON,[1] AND EROL FIKRIG[4]

Department of Entomology, Connecticut Agricultural Experiment Station, New Haven, Connecticut 06504[1]; Section of Immunobiology and Howard Hughes Medical Institute,[2] and Section of Rheumatology, Department of Internal Medicine,[4] Yale University School of Medicine, New Haven, Connecticut 06520; and Division of Rheumatic Diseases, Department of Medicine, University of Connecticut Health Center, Farmington, Connecticut 06030[3]

Received 9 May 1996/Returned for modification 2 July 1996/Accepted 15 October 1996

Serum samples from dogs and equids suspected of having canine or equine borreliosis, respectively, were analyzed in polyvalent enzyme-linked immunosorbent assays (ELISAs) with whole-cell or recombinant antigens of *Borrelia burgdorferi* sensu stricto. Purified preparations of recombinant antigens included outer surface protein A (OspA), OspB, OspC, OspE, OspF, and p41-G (a fragment of flagellin). Of the 36 dog sera that reacted positively to whole-cell antigen, 32 (88.9%) contained antibodies to one or more recombinant antigens. Reactivities to OspF (88.9% positive) and p41-G (75% positive) were most prevalent. In analyses of 30 equid sera positive in an ELISA with whole cells, 24 (80%) contained antibodies to one or more recombinant antigens. Seropositivities in ELISAs with p41-G (50% positive) and OspF (46.7% positive) were more than twofold greater than in ELISAs with OspA, OspB, or OspC (10 to 20% positive). In parallel tests of eight canine and three equine sera, there was good agreement in results of Western blot (immunoblot) analyses and ELISAs. Although dog and equid sera with antibodies to whole-cell *B. burgdorferi* frequently reacted positively to one or more recombinant antigens, the inclusion of OspF and p41-G antigens in ELISAs was most useful in the serologic diagnosis of canine and equine borreliosis.

Lyme borreliosis is prevalent in tick-infested areas of the northeastern and upper midwestern United States. Thousands of human cases have been documented there and elsewhere (1, 4, 27). Dogs and equids can be infected with *Borrelia burgdorferi*, the etiologic agent of canine and equine borreliosis, following feedings by infected *Ixodes scapularis* ticks. Although signs of borreliosis in dogs and equids are more difficult to diagnose than in humans, serologic confirmation of *B. burgdorferi* infection can be made (2, 3, 12–14, 17, 24). Enzyme-linked immunosorbent assays (ELISAs) and Western blot (immunoblot) analyses are relied on frequently to detect class-specific or total immunoglobulins (Ig) to this bacterium. False-positive test results can occur when whole-cell antigen is used (8, 10, 14, 26). An ELISA allows rapid screening of sera and quantitation of antibody concentrations (i.e., titration endpoints), while Western blot analysis, often used as an adjunct method, can show specific banding patterns in response to key immunodominant proteins. However, immunoblotting techniques are laborious and expensive and sometimes yield inconclusive results if key banding patterns are weak or lacking. Newly developed ELISAs containing highly specific and purified recombinant antigens of *B. burgdorferi* have improved laboratory confirmation of this spirochetosis in the diagnosis of human Lyme borreliosis (9, 19, 22). The purpose of our study was to test dog and equid sera, obtained from animals prior to the availability of vaccines against *B. burgdorferi*, to determine if ELISAs with selected recombinant antigens can aid in the laboratory diagnosis of canine and equine borreliosis.

* Corresponding author. Mailing address: Department of Entomology, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, CT 06504-1106. Phone: (203) 789-7241. Fax: (203) 789-7232.

MATERIALS AND METHODS

Collection of blood. The dog and equid serum samples used in this study were obtained by veterinarians in the states of Connecticut, Massachusetts, New York, and Rhode Island from 1984 to 1991. Veterinarians strongly suspected that 55 dogs and 61 equids had been exposed to ticks and had *B. burgdorferi* infections. In many instances, there was evidence of tick bites by *I. scapularis* and signs of illness, such as limb or joint disorders, gait abnormalities, fever, lethargy, and inappetence during the summer or fall. None of the animals included in this study received vaccines for Lyme borreliosis. Further details of clinical findings, the sources of sera, and results of initial serologic tests (including specificity studies) with whole-cell antigens have been reported (14–17, 20). The dog and equid sera selected for analyses in the present study had been stored at −60°C at the Connecticut Agricultural Experiment Station. Positive controls, used earlier in ELISAs (14, 17), consisted of eight dog sera and three equid sera that contained antibodies to outer surface protein A (OspA), a specific polypeptide common to North American strains of *B. burgdorferi* sensu stricto, or other key protein antigens. Reactivity to one or more proteins of this bacterium with molecular masses of 19.2 to 34 kDa was verified by performing Western blot analyses (8). Negative dog sera ($n = 30$) and equid sera ($n = 26$) were obtained from areas of Wyoming and Kent, Connecticut, respectively, where *I. scapularis* is rare or absent. Human cases of Lyme borreliosis were unreported in Wyoming and rare in Kent when dog and equid sera were collected from 1985 to 1987.

Test antigens. Whole-cell and purified preparations of six recombinant antigens, OspA (31 kDa), OspB (34 kDa), OspC (23 kDa), OspE (19 kDa), OspF (29 kDa), and p41-G (the 13-kDa central fragment of flagellin), of *B. burgdorferi* were used separately in polyvalent ELISAs to verify canine or equine borreliosis. These molecular masses represent the recombinant antigens. The glutathione fusion partner attached to each of these proteins has a molecular mass of 26 kDa. The whole-cell antigen (strain 2591) was prepared from live cultures at the Connecticut Agricultural Experiment Station. All recombinant antigens were cloned and expressed as fusion proteins in *Escherichia coli*. The gene encoding OspC was cloned from *B. burgdorferi* 2591 at the University of Connecticut (23), while the remaining recombinant antigens were produced from strain N40 at Yale University (6, 7, 11, 21). The p41-G antigen, a fragment of flagellin (41 kDa), has a selected epitope comprised of amino acids 197 to 273. Since all

TABLE 1. Reactivities of dog sera to whole cells and recombinant antigens of *B. burgdorferi* in a polyvalent ELISA for total Ig

Antigen	No. (%) of serum samples reacting positively to indicated antigen with:	
	Positive sera[a]	Negative sera[b]
OspA	4 (11.1)	0
OspB	3 (8.3)	0
OspC	3 (8.3)	1 (5.3)
OspE	9 (25)	2 (10.5)
OspF	32 (88.9)	1 (5.3)
p41-G	27 (75)	1 (5.3)

[a] Each datum is the number of serum samples with antibodies to the indicated recombinant antigen/36 (the total number of sera reacting positively to whole-cell antigen).

[b] Each datum is the number of serum samples with antibodies to the indicated recombinant antigen/19 (the number of total sera reacting negatively to whole-cell antigen).

recombinant antigens consisted of purified glutathione transferase fusion proteins, affinity-purified glutathione transferase was included as a control in the ELISAs as described before (18) to check for false-positive reactions.

Serologic tests. Concentrations of total Ig to whole-cell or recombinant antigens of *B. burgdorferi* were determined for test sera by polyvalent ELISAs, while class-specific ELISAs for IgG antibodies were used in specificity tests. The materials and procedures used to analyze dog and equid sera have been described before (14, 17). Antigen concentrations ranging from 2 to 5 μg of protein per ml were most suitable for optimal reactivity to recombinant and whole-cell antigens with known positive sera. In polyvalent tests with recombinant antigens, cutoff values for positive results were determined by analyzing 12 negative dog sera and 16 negative equid sera. Net optical density (OD) values were computed for serum dilutions of 1:160, 1:320, and 1:640 or higher by screening the negative sera. Statistical analyses (3 standard deviations plus the mean) of net absorbance values for the respective data sets were conducted to determine values for the critical regions. For dogs, net OD values at three serum dilutions of 0.12, 0.10, and 0.05 were considered positive when OspA was used as the antigen. Cutoff values were comparable for ELISAs with OspB (0.14, 0.09, and 0.04), OspC (0.13, 0.08, and 0.08), OspE (0.15, 0.12, and 0.05), and p41-G (0.08, 0.04, and 0.04). The cutoff value for both polyvalent and class-specific ELISAs with recombinant OspF antigen was 0.04 at all serum dilutions. In a class-specific ELISA, goat serum replaced horse serum in blocking solutions. OD values of critical regions for whole cells (0.19, 0.12, and 0.12) were higher than those calculated for OspA (0.06, 0.04, and 0.04), OspB (0.05, 0.04, and 0.04), OspC (0.09, 0.04, and 0.04), OspE (0.09, 0.05, and 0.05), and p41-G (0.09, 0.05, and 0.05) antigens. In analyses of equid sera, cutoff values in polyvalent ELISAs with p41-G (0.22, 0.12, and 0.16), OspC (0.25, 0.16, and 0.16), and OspE (0.21, 0.18, and 0.12) were higher than those computed in ELISAs with OspA (0.18, 0.12, and 0.07), OspB (0.13, 0.08, and 0.08), and OspF (0.09, 0.05, and 0.04). Net OD values for positive equid results in a class-specific ELISA were higher for whole-cell antigen (0.21, 0.09, and 0.09) than those of critical regions computed for the following recombinant antigens: OspA (0.04, 0.04, and 0.04), OspC (0.04, 0.04, and 0.04), OspE (0.05, 0.04, and 0.04), OspF (0.04, 0.04, and 0.04), and p41-G (0.05, 0.04, and 0.04). The cutoff values for OspB (0.16, 0.08, and 0.08) were comparable to those computed for this antigen in a polyvalent ELISA.

Polystyrene plates contained the same positive and negative control sera, the same controls for phosphate-buffered saline solutions, and peroxidase-labeled antibodies. In addition, murine monoclonal antibodies to OspA (H5332), OspB (H6831), OspC, and flagellin (H9724) of *B. burgdorferi* were used as described before (19) with a 1:3,000 dilution of peroxidase-conjugated goat anti-mouse antibodies in an ELISA to verify antigen reactivity. Immunoblotting of selected positive dog and equid sera confirmed the presence of antibodies to OspE and OspF antigens.

Specificity tests. When whole-cell *B. burgdorferi* was used in ELISAs or indirect fluorescent antibody staining tests to assess specificities of dog and equid sera, there was little or no cross-reactivity with antibodies to *Ehrlichia canis*, *Ehrlichia risticii*, or *Leptospira* species (14, 15). Similarly, antibodies to *E. canis* and *Ehrlichia equi*, the etiologic agents of human and equine granulocytic ehrlichiosis, did not cross-react when tested with respective heterologous antigens (20). Dog and equid sera with (1 specimen each) or without (12 dog specimens and 34 equid specimens) IgG antibodies to *E. equi* were screened in an ELISA containing whole-cell and recombinant antigens of *B. burgdorferi* to further check specificity. The positive sera were from a dog naturally infected by and a horse inoculated with *E. equi* (20). Affinity-purified, horseradish peroxidase-labeled goat anti-dog IgG (γ chain specific) or horseradish peroxidase-conjugated goat anti-horse IgG (γ chain specific) reagents were purchased (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) and diluted to 1:12,000 and 1:3,000, respectively, in phosphate-buffered saline solution for use in ELISAs. In addition, six dog and three equid sera containing antibodies to *B. burgdorferi* were analyzed by indirect fluorescent antibody staining methods for reactivity to *E. equi*. Previous work with human sera (19) demonstrated relatively high specificities for all Osp recombinant antigens of *B. burgdorferi* used in this study.

Immunoblots. Information on immunoblotting procedures for the eight dog and three equid positive control sera has been reported previously (8). Briefly, 1.5 μg of whole-cell *B. burgdorferi* (strain 2591) and 500 ng of separate recombinant antigens were used in gel electrophoresis procedures.

RESULTS

Dog and equid sera with antibodies to whole-cell *B. burgdorferi* also reacted positively in polyvalent ELISAs containing recombinant antigens. Of the 36 dog sera positive when whole-cell antigen was used, 32 (88.9%) contained antibodies to one or more recombinant antigens. Reactivities to OspF (88.9% positive) and p41-G (75%) were most prevalent (Table 1). When 19 dog sera, nonreactive in an ELISA with whole-cell *B. burgdorferi*, were retested with recombinant antigens, 4 (21.1%) had antibodies to OspC, OspE, OspF, or p41-G antigen. One sample contained Ig to OspE and p41-G. Similar results were recorded when equid sera were analyzed. In analyses of 30 equid sera that reacted positively to whole-cell antigen, 24 (80%) also contained antibodies to one or more recombinant antigens. Paralleling the findings for dog sera, reactivities to p41-G (50%) and OspF (46.7%) were most prevalent (Table 2). Seropositivities to OspA, OspB, and OspC ranged between 10 and 20%. By contrast, 16 of 31 (51.6%) equid sera reacted positively to recombinant antigens but were negative in an ELISA with whole-cell antigens. Numbers of sera that reacted positively to OspA and OspE were nearly twofold greater than those of the remaining sera that had antibodies to other recombinant antigens. Positive reactions to two or more recombinant antigens were noted for 12 sera.

Antibody titers for positive dog sera were usually higher than those recorded for equid sera when whole cells or certain recombinant antigens were used in ELISAs. Geometric mean antibody titers for dog sera tested with whole-cell, p41-G, and OspF antigens were twofold or more higher than those computed for equid sera with these antigens (Table 3). Relatively low titration endpoints (1:160 to 1:640) were recorded for dog and equid sera when OspB and OspC were included in analyses.

Patterns of reactivity to whole-cell and recombinant antigens were highly variable for positive dog and equid sera. In most instances, reactivities to whole-cell *B. burgdorferi* and to one or more recombinant antigens occurred in separate tests (Table 4). Detection of antibodies to whole cells, p41-G, and to one or more Osp antigens was most frequent. By contrast, 5 dog sera and 16 equid sera contained Ig to one or more recombinant

TABLE 2. Reactivities of equid sera to whole-cell and recombinant antigens of *B. burgdorferi* in a polyvalent ELISA for total Ig

Antigen	No. (%) of serum samples reacting positively to indicated antigen with:	
	Positive sera[a]	Negative sera[a]
OspA	6 (20)	10 (32.3)
OspB	3 (10)	2 (6.5)
OspC	6 (20)	2 (6.5)
OspE	9 (3.3)	9 (29)
OspF	14 (46.7)	3 (9.7)
p41-G	15 (50)	4 (12.9)

[a] See the footnotes to Table 1 for calculation procedures. The total number of sera reacting positively to whole-cell *B. burgdorferi* was 30; the total number of sera reacting negatively to whole-cell antigen was 31.

TABLE 3. Geometric means and ranges of titers of antibodies to whole-cell and recombinant antigens of *B. burgdorferi* in polyvalent ELISAs for dog and equid sera

Antigen	Total no. of positive dog sera (n = 55)	Dog antibody titer		No. of positive equid sera (n = 61)	Equid antibody titer	
		Geometric mean[a]	Range		Geometric mean	Range
Whole cells	36	1,143	320–40,960	30	256	640–20,480
p41-G	28	246	160–5,120	19	100	160–2,560
OspA	4	48	160–10,240	16	81	160–640
OspB	3	44	160–320	5	49	320–640
OspC	4	46	160–320	8	52	160–640
OspE	11	62	160–1,280	18	82	160–640
OspF	33	571	160–40,960	16	99	160–5,120

[a] A value of 40 (average reciprocal titer for a negative serum sample) was used to compute geometric means for sera lacking antibodies to *B. burgdorferi.*

antigens but were negative in ELISAs with whole-cell *B. burgdorferi*.

Results of Western blot analyses and ELISAs were compared. When whole-cell lysates were tested with eight canine and three equine positive control serum samples, there were antibodies to multiple proteins with molecular masses ranging from 17 to 41 kDa. Banding patterns in the immunoblots indicated distinct positive reactions to several polypeptides, including OspE (19 kDa) and OspF (29 kDa). Results for canine sera, tested by ELISAs with separate recombinant antigens, confirmed antibody reactions to OspA (n = 3), OspC (n = 2), OspE (n = 4), OspF (n = 8), and p41-G (n = 8). In similar analyses of three equid sera, positive immunoblots were confirmed by ELISAs. There were antibody reactions to OspA (n = 2), OspB (n = 1), OspC (n = 1), OspE (n = 1), OspF (n = 2), and p41-G (n = 3).

In specificity tests, one dog and one equid serum sample with IgG antibodies to *E. equi* were tested in class-specific ELISAs containing whole-cell or recombinant antigens of *B. burgdorferi*. There was weak reactivity of the dog serum to whole-cell antigens at a dilution of 1:160. The remaining results for the dog and equid sera with whole-cell and recombinant antigens were negative. When six dog and three equid sera containing antibodies to *B. burgdorferi* (as determined by ELISA and Western blot analyses) were analyzed by indirect fluorescent antibody staining methods with *E. equi* antigen, results were negative. All homologous reactions were positive at titers of 1:1,280 or higher, and the five negative control sera for each animal species were nonreactive in all tests.

DISCUSSION

Multiple recombinant antigens of *B. burgdorferi* can be used separately in ELISAs for serologic diagnosis of canine or equine borreliosis. Compared to tests with whole cells, results of ELISAs with recombinant Osp antigens yielded more relevant information by indicating the presence of antibodies to individual, highly specific antigens. Also, there was good agreement when results of ELISAs and immunoblotting were compared. Assay sensitivities in ELISAs were particularly high when p41-G and OspF antigens were included in the screening of canine and equine sera. As in tests with human sera (19), reactivities of dog and equid sera to OspA, OspB, and OspE were infrequent. In other studies, a specific antibody response to OspA was noted during early infection (25) and dogs immunized with recombinant OspA vaccine prior to challenge with infected ticks produced high antibody titers (29). Clearly, immune responses by dogs and equids are variable and several factors, including vaccination, should be considered when interpreting serologic results.

There were notable differences in the reactivities of human, dog, and equid sera to OspC. In the early stage of Lyme borreliosis, there is marked IgM antibody response to OspC in human sera (9, 19, 22, 30, 31). The number of canine and equine sera with antibodies to OspC in our study was low. *Borrelia* infections in these animals may not have been recent, and consequently, the detected antibodies to the other Osps could have been predominantly in the IgG classes. In murine and human infections of *B. burgdorferi*, production of antibody to OspF usually occurs later in the course of the disease (21), when there is an expansion in immune response. In Europe, where different strains of *B. burgdorferi* sensu lato occur, results varied when recombinant OspC and other key immunodominant antigens of this spirochete were tested with human sera by Western blot analysis (30, 31). Further studies of the use of recombinant antigens in class-specific ELISAs are needed to quantitate concentrations of IgM and IgG antibodies in dogs and equids. Other key recombinant antigens, such as p39 and p93, should be evaluated.

The use of vaccines that contain whole-cell *B. burgdorferi* or the recombinant OspA component of this bacterium can complicate serologic diagnosis of Lyme borreliosis if conventional assays with whole-cell antigen are utilized. There was a broad host immune response in domestic animals when the whole-cell vaccine was used, while humoral responses in mice and dogs which received the recombinant OspA vaccine were limited to OspA antigen (6, 29). Only the more refined recombi-

TABLE 4. Summary of ELISA results for dog and equid sera after testing for antibodies to whole-cell or recombinant antigens of *B. burgdorferi*

Antigen(s)	Total no. of sera with antibodies to indicated antigen(s)	
	Dog	Equid
Whole cells only	4	6
Whole cells and p41-G only	0	2
Whole cells, p41-G, and one or more Osps	27[a]	13[b]
Whole cells and one or more Osps	5[c]	9[d]
p41-G and one or more Osps	1	4
One or more Osps	0	5
Single Osp	3	7[e]
None	15	15

[a] Thirteen sera had antibodies to whole cells, p41-G, and OspF only.
[b] Twelve sera had antibodies to OspA and/or OspF.
[c] All samples had antibodies to OspF.
[d] Seven sera had antibodies to OspE and/or OspF.
[e] Sera had antibodies to OspA (n = 3), OspB (n = 1), OspE (n = 2), or OspF (n = 1).

nant OspA vaccines are currently being evaluated in human clinical trials. If these vaccines are accepted for widespread use and the recipients have had no prior exposure to *B. burgdorferi*, then ELISAs and immunoblots that contain recombinant OspA antigen may be useful for verifying immune response to this polypeptide. Without vaccination, assays with one or more key recombinant antigens may help determine if there was natural exposure to *B. burgdorferi*. Immunoblots of naturally infected dogs, including those tested in our study, showed major and minor bands reactive to several proteins (10). Therefore, the selection of recombinant antigens for use in ELISAs or Western blot analysis will depend on the history of host exposure to *B. burgdorferi*. Without such information, more extensive laboratory testing may be needed.

Some dog and equid sera reacted positively to one or more recombinant antigens but were negative when tested with whole-cell *B. burgdorferi*. This has been observed before when white-footed mouse (*Peromyscus leucopus*), raccoon (*Procyon lotor*), and human sera were tested (16, 19). When polystyrene plates are coated with whole-cell antigen, many proteins and other components of *B. burgdorferi* (not frequently recognized immunologically by mammalian hosts) also bind to the immunoplates. With the less important spirochetal components present, there can be inadequate formation of the more specific antibody-antigen complexes. Consequently, assay sensitivity can decrease. Although host immune responses to *B. burgdorferi* are highly variable (10, 27, 30, 31), production of antibody to key antigens occurs frequently when IgG antibodies are developed. Therefore, key recombinant antigens can be used instead of whole-cell sonicates to enhance optimal and more specific antibody-antigen complex formation and, thereby, improve assay sensitivity.

The separate use of recombinant antigens of *B. burgdorferi* in ELISAs shows promise in laboratory diagnosis. Similar applications of these reagents instead of whole-cell lysates in Western blot analysis might reduce technical problems associated with comigration of different proteins (having similar molecular masses) to the same area and with the formation of weak specific banding patterns. The latter can result if there is interference (i.e., blocking) caused by the activity of competing nonspecific antibodies. Ultimately, the recombinant antigens chosen should detect antibodies produced in response to highly specific immunodominant polypeptides of *B. burgdorferi*. Host immune responses vary within and among animal species, and there are different strains of *B. burgdorferi* in nature (30). Therefore, the selection of particular recombinant antigens for diagnostic purposes should be based on knowledge of the most common patterns of antibody production following natural exposure to *B. burgdorferi*. When interpreting test results, consideration should also be given to Osp antigens known to be differently expressed, such as OspA, OspC, OspE, and OspF (5, 28). Antibodies to one or more of these antigens may not always be produced in natural infections. Nonetheless, of the recombinant antigens evaluated in this study, OspF and p41-G antigens seemed particularly useful in the serologic diagnosis of canine and equine borreliosis.

ACKNOWLEDGMENTS

We thank Tia Blevins, Bonnie Hamid, Manchuan Chen, and Hong Tao for their assistance in preparing antigens and performing analyses. Alan G. Barbour of the University of Texas (San Antonio) and Bettina Wilske of the University of Munich (Germany) kindly provided monoclonal antibodies.

This work was supported in part by grants from the Centers for Disease Control and Prevention (CCU-106581), the National Institutes of Health (PO-1-AI-30548 and AI-49988), the Mathers Foundation, the Arthritis Foundation, the American Heart Association, and the State of Connecticut (Charles Goodyear Award). Erol Fikrig is a Pew Scholar and Richard A. Flavell is an investigator in the Howard Hughes Medical Institute.

REFERENCES

1. **Cartter, M. L., P. Mshar, and J. L. Hadler.** 1989. The epidemiology of Lyme disease in Connecticut. Conn. Med. **53:**320–323.
2. **Cohen, N. D., and D. Cohen.** 1990. Borreliosis in horses: a comparative review. Compend. Practicing Vet. **12:**1449–1458.
3. **Cohen, N. D., F. C. Heck, B. Heim, D. M. Flad, E. M. Bosler, and D. Cohen.** 1992. Seroprevalence of antibodies to *Borrelia burgdorferi* in a population of horses in central Texas. J. Am. Vet. Med. Assoc. **201:**1030–1034.
4. **Dennis, D. T.** 1995. Lyme disease. Dermatol. Clin. **13:**537–551.
5. **DeSilva, A. M., S. R. Telford III, L. R. Brunet, S. W. Barthold, and E. Fikrig.** 1996. *Borrelia burgdorferi* OspA is an arthropod-specific transmission-blocking Lyme disease vaccine. J. Exp. Med. **183:**271–275.
6. **Fikrig, E., S. W. Barthold, and R. A. Flavell.** 1993. OspA vaccination of mice with established *Borrelia burgdorferi* infection alters disease but not infection. Infect. Immun. **61:**2553–2557.
7. **Fikrig, E., S. W. Barthold, N. Marcantonio, K. DePonte, F. S. Kantor, and R. A. Flavell.** 1992. Roles of OspA, OspB, and flagellin in protective immunity to Lyme borreliosis in laboratory mice. Infect. Immun. **60:**657–661.
8. **Fikrig, E., L. A. Magnarelli, M. Chen, J. F. Anderson, and R. A. Flavell.** 1993. Serologic analyses of dogs, horses, and cottontail rabbits for antibodies to an antigenic flagellar epitope of *Borrelia burgdorferi*. J. Clin. Microbiol. **31:**2451–2455.
9. **Gerber, M. A., E. D. Shapiro, G. L. Bell, A. Sampieri, and S. J. Padula.** 1995. Recombinant outer surface protein C ELISA for the diagnosis of early Lyme disease. J. Infect. Dis. **171:**724–727.
10. **Greene, R. T., R. L. Walker, W. L. Nicholson, H. W. Heidner, J. F. Levine, E. C. Burgess, M. Wyand, E. B. Breitschwerdt, and H. A. Berkhoff.** 1988. Immunoblot analysis of immunoglobulin G to the Lyme disease agent (*Borrelia burgdorferi*) in experimentally and naturally infected dogs. J. Clin. Microbiol. **26:**648–653.
11. **Lam, T. T., T.-P. K. Nguyen, R. R. Montgomery, F. S. Kantor, E. Fikrig, and R. A. Flavell.** 1994. Outer surface proteins E and F of *Borrelia burgdorferi*, the agent of Lyme disease. Infect. Immun. **62:**290–298.
12. **Levy, S. A., S. W. Barthold, D. M. Daubach, and T. L. Wasmoen.** 1993. Canine Lyme borreliosis. Compend. Practicing Vet. **15:**833–848.
13. **Levy, S. A., and L. A. Magnarelli.** 1992. Relationship between development of antibodies to *Borrelia burgdorferi* in dogs and the subsequent development of limb/joint borreliosis. J. Am. Vet. Med. Assoc. **200:**344–347.
14. **Magnarelli, L. A., and J. F. Anderson.** 1989. Class-specific and polyvalent enzyme-linked immunosorbent assays for detection of antibodies to *Borrelia burgdorferi* in equids. J. Am. Vet. Med. Assoc. **195:**1365–1368.
15. **Magnarelli, L. A., and J. F. Anderson.** 1993. Serologic evidence of canine and equine ehrlichiosis in northeastern United States. J. Clin. Microbiol. **31:**2857–2860.
16. **Magnarelli, L. A., J. F. Anderson, R. A. Flavell, and E. Fikrig.** 1995. Reactivity of white-footed mouse and raccoon sera to whole cell and recombinant antigens of *Borrelia burgdorferi*. J. Wildl. Dis. **31:**339–344.
17. **Magnarelli, L. A., J. F. Anderson, and A. B. Schreier.** 1990. Persistence of antibodies to *Borrelia burgdorferi* in dogs of New York and Connecticut. J. Am. Vet. Med. Assoc. **196:**1064–1068.
18. **Magnarelli, L. A., E. Fikrig, R. Berland, J. F. Anderson, and R. A. Flavell.** 1992. Comparison of whole-cell antibodies and an antigenic flagellar epitope of *Borrelia burgdorferi* in serologic tests for diagnosis of Lyme borreliosis. J. Clin. Microbiol. **30:**3158–3162.
19. **Magnarelli, L. A., E. Fikrig, S. J. Padula, J. F. Anderson, and R. A. Flavell.** 1996. Use of recombinant antigens of *Borrelia burgdorferi* in serologic tests for diagnosis of Lyme borreliosis. J. Clin. Microbiol. **34:**237–240.
20. **Magnarelli, L. A., K. C. Stafford III, T. N. Mather, M.-T. Yeh, K. D. Horn, and J. S. Dumler.** 1995. Hemocytic rickettsia-like organisms in ticks: serologic reactivity with antisera to ehrlichiae and detection of DNA of agent of human granulocytic ehrlichiosis by PCR. J. Clin. Microbiol. **33:**2710–2714.
21. **Nguyen, T.-P. K., T. T. Lam, S. W. Barthold, S. R. Telford III, R. A. Flavell, and E. Fikrig.** 1994. Partial destruction of *Borrelia burgdorferi* within ticks that engorged on OspE- or OspF-immunized mice. Infect. Immun. **62:**2079–2084.
22. **Padula, S. J., F. Dias, A. Sampieri, R. B. Craven, and R. W. Ryan.** 1994. Use of recombinant OspC from *Borrelia burgdorferi* for serodiagnosis of early Lyme disease. J. Clin. Microbiol. **32:**1733–1738.
23. **Padula, S. J., A. Sampieri, F. Dias, A. Szczepanski, and R. W. Ryan.** 1993. Molecular characterization and expression of p23 (OspC) from a North American strain of *Borrelia burgdorferi*. Infect. Immun. **61:**5097–5105.
24. **Parker, J. L., and K. K. White.** 1992. Lyme borreliosis in cattle and horses: a review of the literature. Cornell Vet. **82:**253–274.
25. **Schutzer, S. E., P. K. Coyle, J. J. Dunn, B. J. Luft, and M. Brunner.** 1994. Early and specific antibody response to OspA in Lyme disease. J. Clin. Invest. **94:**454–457.
26. **Shin, S. J., Y. F. Chang, R. H. Jacobson, E. Shaw, T.-L. Lauderdale, M. J. G.**

Appel, and D. H. Lein. 1993. Cross-reactivity between *Borrelia burgdorferi* and other spirochetes affects specificity of serotests for detection of antibodies to the Lyme disease agent in dogs. Vet. Microbiol. **36:**161–174.
27. **Steere, A. C.** 1989. Lyme disease. N. Engl. J. Med. **321:**586–596.
28. **Stevenson, B., T. G. Schwan, and P. A. Rosa.** 1995. Temperature-related differential expression of antigens in the Lyme disease spirochete, *Borrelia burgdorferi*. Infect. Immun. **63:**4535–4539.
29. **Straubinger, R. K., Y.-F. Chang, R. H. Jacobson, and M. J. G. Appel.** 1995. Sera from OspA-vaccinated dogs, but not those from tick-infested dogs, inhibit in vitro growth of *Borrelia burgdorferi*. J. Clin. Microbiol. **33:**2745–2751.
30. **Wilske, B., V. Fingerle, P. Herzer, A. Hofmann, G. Lehnert, H. Peters, H.-W. Pfister, V. Preac-Mursic, E. Soutschek, and K. Weber.** 1993. Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. Med. Microbiol. Immunol. **182:**255–270.
31. **Wilske, B., V. Fingerle, V. Preac-Mursic, S. Jauris-Heipke, A. Hofmann, H. Loy, W.-W. Pfister, D. Rossler, and E. Soutschek.** 1994. Immunoblot using recombinant antigens derived from different genospecies of *Borrelia burgdorferi* sensu lato. Med. Microbiol. Immunol. **183:**43–59.