

# UC Irvine

## UC Irvine Previously Published Works

### Title

Diversity of Antibody Responses to *Borrelia burgdorferi* in Experimentally Infected Beagle Dogs

### Permalink

<https://escholarship.org/uc/item/2c5789r4>

### Journal

mSphere, 21(6)

### ISSN

1556-6811

### Authors

Baum, Elisabeth  
Grosenbaugh, Deborah A  
Barbour, Alan G

### Publication Date

2014-06-01

### DOI

10.1128/cvi.00018-14

Peer reviewed

# Diversity of Antibody Responses to *Borrelia burgdorferi* in Experimentally Infected Beagle Dogs

Elisabeth Baum,<sup>a\*</sup> Deborah A. Grosenbaugh,<sup>b</sup> Alan G. Barbour<sup>a</sup>

Departments of Microbiology and Molecular Genetics and Medicine, University of California, Irvine, Irvine, California, USA<sup>a</sup>; Merial Inc., Athens, Georgia, USA<sup>b</sup>

Lyme borreliosis (LB) is a common infection of domestic dogs in areas where there is enzootic transmission of the agent *Borrelia burgdorferi*. While immunoassays based on individual subunits have mostly supplanted the use of whole-cell preparations for canine serology, only a limited number of informative antigens have been identified. To more broadly characterize the antibody responses to *B. burgdorferi* infection and to assess the diversity of those responses in individual dogs, we examined sera from 32 adult colony-bred beagle dogs that had been experimentally infected with *B. burgdorferi* through tick bites and compared those sera in a protein microarray with sera from uninfected dogs in their antibody reactivities to various recombinant chromosome- and plasmid-encoded *B. burgdorferi* proteins, including 24 serotype-defining OspC proteins of North America. The profiles of immunogenic proteins for the dogs were largely similar to those for humans and natural-reservoir rodents; these proteins included the decorin-binding protein DbpB, BBA36, BBA57, BBA64, the fibronectin-binding protein BBK32, VlsE, FlaB and other flagellar structural proteins, Erp proteins, Bdr proteins, and all of the OspC proteins. In addition, the canine sera bound to the presumptive lipoproteins BBB14 and BB0844, which infrequently elicited antibodies in humans or rodents. Although the beagle, like most other domestic dog breeds, has a small effective population size and features extensive linkage disequilibrium, the group of animals studied here demonstrated diversity in antibody responses in measures of antibody levels and specificities for conserved proteins, such as DbpB, and polymorphic proteins, such as OspC.

In North America, Lyme borreliosis (LB) is caused by the spirochete *Borrelia burgdorferi*, which is transmitted during the blood meal by the hard tick *Ixodes scapularis* in the Northeast and upper Midwest regions. Because of their outdoor activities and body fur, dogs are generally at higher risk of tick bites and tick-borne diseases than humans (1). Immunoassays of serum samples from pet dogs revealed positive correlations between the prevalence of antibodies to *B. burgdorferi* antigens and the distribution of *I. scapularis* (2, 3). In addition, dogs serve as a sentinel species for surveillance programs and provide estimates of the risk to humans in a given region (4–6).

Most adult dogs with naturally acquired *B. burgdorferi* infection do not show signs of illness (7–9). Among the minority of dogs with symptoms, recurrent lameness is the most common presenting manifestation (8–10). With experimental infections through needle inoculation or tick bites, puppies 6 to 12 weeks of age were much more likely to have symptoms than were dogs infected at  $\geq 6$  months of age. Almost all dogs who were experimentally infected, as documented by culture and/or PCR, exhibited seroconversion to expression of *B. burgdorferi*-specific antigens in the studies (8, 11–13).

In the past, immunoassays for canine infections generally applied the same protocol as used for human infections, namely, a two-tier approach in which a whole-cell-based, enzyme-linked immunosorbent assay (ELISA) was conditionally followed by a Western blotting (WB) assay (14, 15). More recently, immunoassays based on whole cells have been supplanted by assays based on either the C6 peptide of the VlsE protein of *B. burgdorferi* or a small set of purified antigens (14). One potential drawback of assays based on the VlsE protein is its homology to the variable membrane proteins (VMPs) of relapsing-fever *Borrelia* spp., for which it is named (“VMP-like sequence”) (16). Reports of human infections by *Borrelia miyamotoi*, a member of the relapsing-fever group of species, in areas with *B. burgdorferi* transmission mean

that interpretation of VlsE-based assays may be confounded by hitherto unrecognized cross-reactivity between *B. miyamotoi* and *B. burgdorferi*, as Krause et al. suggested (17).

Previous studies of recombinant proteins as candidate subunits for serological assays were limited to a single antigen or a small number of antigens at a time (18–22). To characterize more broadly the antibody responses of dogs with infections, we examined sera from a group of dogs that had been experimentally infected with *B. burgdorferi*, with microarrays containing several recombinant *B. burgdorferi* proteins, most of which were immunogenic for either or both humans and *Peromyscus leucopus* rodents (23, 24), as well as different types of OspC proteins (25), major serotype-defining antigens (26). The protein arrays allowed the evaluation of the canine antibody responses to several proteins simultaneously, which allowed for not only quantitation but also resolution of individual proteins.

Our first aim was to explore more fully the breadth of antibody responses of experimentally infected dogs by using a large set of proteins. This would provide for comparisons with the immune

Received 9 January 2014 Returned for modification 12 February 2014

Accepted 28 March 2014

Published ahead of print 2 April 2014

Editor: P. P. Wilkins

Address correspondence to Elisabeth Baum, ebaum@uci.edu, or Alan G. Barbour, abarbour@uci.edu.

\* Present address: Elisabeth Baum, Division of Infectious Diseases, Department of Medicine, University of California Irvine, Irvine, California, USA.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/CVI.00018-14>.

Copyright © 2014, American Society for Microbiology. All Rights Reserved.

doi:10.1128/CVI.00018-14

responses of humans, natural hosts (such as *Peromyscus leucopus*), and laboratory mice. A long-term goal is improved immunoassays for laboratory confirmation of LB in dogs. The study's second major aim was to assess the diversity of antibody responses within a single canine breed infected and monitored under the same conditions. The stratified, breed-based, population structure of *Canis lupus familiaris* provides a suitable background for genomic analysis of complex traits or diseases (27, 28), such as a genome-wide association study (GWAS) of host responses to infection or immunization. Colony-bred and -reared beagle dogs are a common experimental model of LB (8, 29–31), the beagle genome sequence is nearly complete (32), and single-nucleotide polymorphism (SNP) genotyping chips for dogs are available (33, 34). However, quantitative trait locus mapping and GWAS will be most informative with phenotypes that are well defined and relevant for infection outcomes or vaccination success.

## MATERIALS AND METHODS

**Serum samples.** Serum samples were leftover specimens from studies involving experimental tick infections that were conducted in 2006 by Merial Inc. (Athens, GA). Animals were managed similarly and were handled in compliance with the local institutional animal care and use committee (IACUC) guidelines at Merial and with IACUC approval of the protocol. Additional justifications for the test system and model, the number of animals, and the routes of administration were defined in an approved animal procedure statement (animal procedure statement R 05-93M-06/06, as amended in R 06-85M-07/07) for the IACUC. The subjects were colony-bred non-barrier-reared intact beagle dogs (from Ridgeman Farms, Mount Horeb, WI) of both sexes, 8 to 15 months of age at the beginning of the study. The heat cycles of female dogs were not monitored or synchronized. The dogs had been vaccinated against *Bordetella bronchiseptica*, canine adenovirus type 2, canine parainfluenza virus, canine distemper, and canine parvovirus as puppies and were negative for intestinal worms when they were received at the facility. Occasional minor eye and ear infections that occurred during the experimental period were treated with nonsteroidal anti-inflammatory drugs and topical antibiotics as needed. No other drugs were administered to the dogs during this period.

Field-collected adult *I. scapularis* ticks for the infections were provided by Thomas Mather (University of Rhode Island) and were drawn from a population with a *B. burgdorferi* infection prevalence of ~50% in adult ticks (35). On day 208 or 412 of the study (Table 1), 13 female and 12 male *I. scapularis* ticks were confined in a 5-cm-diameter plastic capsule secured on the thorax of each dog. Ticks were allowed to feed to repletion and detachment over a 4- to 7-day period, during which the dogs were individually housed.

The serum samples were from subjects of a preclinical study to evaluate the effects of alternative routes of vaccine administration and different adjuvants on the immunogenicity of recombinant OspA protein of *B. burgdorferi*. None of the vaccines had the same formulation or route of administration as the current commercial product offered by Merial Limited Inc. Specific information regarding the experimental vaccine formulations is proprietary. Of the 32 samples in the study, 23 were from a subset of subjects that had received one of the experimental protocols 37 weeks or 66 weeks previously (Table 1) but were seronegative for antibodies to OspA, as described below, at the time of tick placement. The other 9 serum samples were from infected unimmunized controls. Serum samples were collected on day 285 or day 496 of the study, i.e., approximately 11 or 12 weeks after tick detachment. There were no clinical signs of disease in the infected dogs during this period. Blood samples were collected via the jugular vein, and individual serum samples were frozen at -20°C. Serum samples obtained from 4 study dogs prior to immunization and tick placement served as negative controls. We also used pooled serum samples

TABLE 1 Characteristics of 32 beagle dogs infected by *I. scapularis* tick bites

Dog	Group <sup>a</sup>	Time (days) <sup>b</sup>	Culture result <sup>c</sup>	PCR assay result <sup>d</sup>	OspF antibody <sup>e</sup>
LXR	Exp	496	+	+	+
LZR	Exp	496	+	+	+
OUQ	Exp	496	+	+	+
PHQ	Exp	285	+	+	+
PKQ	Exp	285	+	+	+
PLQ	Exp	496	+	+	+
PRQ	Exp	496	+	+	+
PTQ	Cont	285	+	+	+
PWR	Exp	285	+	+	+
PXR	Exp	285	+	+	+
QLR	Exp	496	+	+	+
QPQ	Cont	496	+	+	+
QQQ	Exp	496	+	+	+
QRQ	Exp	496	+	+	+
QWQ	Exp	496	+	+	+
VGQ	Exp	496	+	+	+
VIR	Exp	496	+	+	+
VJR	Cont	496	-	+	+
VSR	Exp	496	-	+	+
VTQ	Exp	496	+	+	+
WDQ	Cont	285	+	+	+
WFR	Cont	496	+	-	+
WQR	Exp	496	+	+	+
XFR	Cont	496	+	+	+
XHR	Exp	496	-	+	+
XKR	Exp	285	+	+	+
XTR	Exp	496	-	+	+
XYR	Cont	285	+	+	+
XZQ	Cont	496	+	+	+
YOQ	Exp	285	+	+	+
YYQ	Cont	496	+	+	+
ZEQ	Exp	285	+	+	+

<sup>a</sup> Exp, experimental vaccine group; Cont, control group.

<sup>b</sup> Time indicates the time prior to the final serum sample at which the dog received experimental vaccine or was designated a control animal.

<sup>c</sup> +, *B. burgdorferi* was isolated from ≥1 skin biopsy specimen culture; -, all cultures were negative.

<sup>d</sup> +, *B. burgdorferi* was detected in ≥1 PCR assay of a skin biopsy specimen; -, all PCR assay results were negative.

<sup>e</sup> Antibody to OspF (ErpY) by Western blotting.

from specific-pathogen-free beagle dogs (Innovative Research, Southfield, MI) as an additional negative control.

**Diagnostic procedures.** ELISAs for detection of serum antibodies to OspA (open reading frame [ORF] BBA15 for strain B31) and ErpY, formerly known as OspF (GenBank accession L13925) (ORF BBR42 for strain B31), and immunoblots with whole-cell lysates of *B. burgdorferi* were performed at the Animal Health Diagnostic Center at Cornell University (Ithaca, NY), as described previously (8). The presence of anti-*B. burgdorferi* antibodies was confirmed by array assays with whole-cell lysates, as described below. Infection of the dogs after tick exposure was assessed by PCR assays and culture of skin punch biopsy specimens collected during weeks 4, 8, and 11 after tick exposure, as described by Chang et al. (8). Selected culture isolates were confirmed as *B. burgdorferi* as described previously (36). Sera were not tested for antibodies to *Anaplasma phagocytophilum*, *Bartonella* spp., *Babesia microti*, *B. miyamotoi*, or the Powassan or deer tick viruses.

**Protein microarrays.** The first array included 69 selected *B. burgdorferi* proteins of strain B31 that were produced in *Escherichia coli* *in vitro* transcription-translation systems; this array was described previously for

a study of human sera (23). Six spots containing the *in vitro* coupled transcription-translation mixture but without template DNA were printed as background controls. The first array also included purified preparations of the proteins BB0279 (FliL), BB0283 (FlgE), BB0289 (flagellar assembly protein FliH), and BBA25 (decorin-binding protein B) and the lp36 plasmid-encoded proteins BBK07, BBK12, and BBK19 (23), each printed in duplicate in four dilutions over the range of 30 to 900  $\mu\text{g}$  per ml, in volumes of 1.5 nl per spot. The second array was described previously (24, 25); it contained purified, nonlipidated, recombinant proteins representing 24 different types of OspC from *B. burgdorferi* strains in North America, i.e., A, A3, B, B3, C, C3, D, D3, E, E3, F, F3, I3, G, H3, H, I, J, K, L, M, N, T, and U. The second array also contained purified BB0279, BB0283, BB0383 (P39 or BmpA), BB0774 (P83 antigen), BBA25, BBK07, BBK12, and BBK19, full-length VlsE of strain B31, and a whole-cell lysate of strain B31 (23). The second array also included nonlipidated recombinant OspC type A, I, and K proteins at  $\geq 99\%$  purity (provided by MCLAB, San Francisco, CA). Prediction of lipoproteins and assignments to a paralogous family (PF) of proteins was performed according to an annotation reference for the coding sequences (see <http://www.blackwellpublishing.com/products/journals/suppmat/mole/casjens.htm>).

**Antibody binding.** Sera were diluted 1:250 in protein array blocking (PAB) buffer (Whatman Inc., Sanford, ME), supplemented with 10% (vol/vol) DH5 $\alpha$  *Escherichia coli* lysate (MCLAB, San Francisco, CA) as an absorbent for preexisting antibodies to *E. coli* in the dogs, and incubated for 30 min at 22°C with shaking. After rehydration of the membranes with PAB buffer alone, the membranes were incubated with serum and lysate solution for 12 h at 4°C, on a rocker. Pads were quickly rinsed 6 times with 10 mM Tris (pH 8.0), 150 mM NaCl (Tris-buffered saline [TBS]), with 0.05% Tween 20 (Tween-TBS) and then were washed 3 times for 5 min each time, on a rocking platform. Biotin-conjugated rabbit anti-dog IgG (heavy and light chains; Jackson ImmunoResearch, West Grove, PA) was diluted 1:200 in PAB buffer and incubated with the pads for 1 h at 22°C, on a rocker. Arrays were washed as described above and were incubated with Cy5-conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA), diluted 1:200 in PAB buffer, in the dark for 1 h at 22°C, on a rocker. Arrays were rinsed and washed as described above and finally were washed in double-distilled water. When dry, the arrays were scanned in a PerkinElmer ScanArray Express HT scanner at 670 nm. Output red-green-blue (RGB) TIFF files were quantitated using ProScanArray Express software (PerkinElmer), with spot-specific background correction.

**Data analysis.** The raw values (in pixels) for antibody binding were the averages of duplicate spots for each printed antigen. For purified proteins, the raw values were used for analysis. For antigens produced by *in vitro* coupled transcription-translation reactions, where *E. coli* proteins were present, the background signal for each serum sample was calculated as the mean of the array's 6 spots without input plasmid DNA in the coupled reaction. This was subtracted from each raw value to yield a net value for data analysis for that dog. For logarithmic transformations, a negative net value was assigned a net log value of 0. The following analyses were used: descriptive statistics of pixel intensity, fold differences over uninfected controls, and 2-tailed Student's *t* test. The *Z* score was the number of standard deviations of the control values above or below the control mean for a given experimental value. Standard statistical analyses, including 2-tailed contingency tables, Pearson correlations, coefficients of determination ( $R^2$ ), 2-tailed Mann-Whitney nonparametric rank tests, and generalized linear models, were carried out with Systat v. 11 (Systat Software, Inc.) or Stata v. 10.1 (Stata Corp.). Mean values are presented with 95% confidence intervals (CIs). The binary criterion for positive results for a particular serum with an antigen in the first array was a *Z* score of  $\geq 2$  for 4 control sera, and that for the 24 OspC proteins in the second array was a *Z* score of  $\geq 2.5$  for 120 values for 5 control sera.

**Microarray data accession numbers.** The array data were deposited in the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/gds>) under GEO Series accession number GSE50074 for the partial *B. burgdorferi* proteome array and accession number GSE50075 for the OspC array.

## RESULTS

**Canine infections.** All 32 dogs, including 23 that had been vaccinated several months previously and 9 that had not, became infected by the criterion of  $\geq 1$  positive skin biopsy specimen culture (28 of 32 dogs) and/or positive PCR results (31 of 32 dogs) within 11 or 12 weeks after tick placement (Table 1). All of the 32 dogs had serum antibodies to ErpY (OspF), as determined by Western blotting. By the criterion of a *Z* score of  $\geq 4$ , all of the 32 experimental dogs had antibodies to the whole-cell lysate of *B. burgdorferi* (*Z* score mean, 57 [range, 23 to 77]).

**Antibody binding to 69 *B. burgdorferi* antigens.** In the first array, the mean cumulative pixel intensities per spot of antibody binding were 1,964 pixels per spot (95% CI, 1,537 to 2,510 pixels per spot) for the sera from the 32 infected dogs and 381 pixels per spot (95% CI, 330 to 439 pixels per spot) for the sera from the 4 uninfected dogs ( $P < 0.0001$ ). The mean pixel intensities per spot of antibody binding for all proteins in the array were 2,104 pixels per spot (95% CI, 1,577 to 2,806 pixels per spot) for the vaccine group and 1,705 pixels per spot (95% CI, 1,033 to 2,813 pixels per spot) for the nonvaccine group ( $t$  test  $P = 0.46$ ; Mann-Whitney  $P = 0.53$ ). Figure 1 is a gradient two-color, two-dimensional display of signal intensity values for serum antibody binding to the 69 proteins for the 32 infected and 4 control dogs. The sera are ranked from left to right in their totals of binding to the array's proteins, and the ORF proteins are ranked from top to bottom in their mean values of antibody binding for all sera.

For the individual ORFs, similar rankings were obtained by the criteria of reciprocal of the *t* test *P* value, frequencies of positive sera, fold difference, and *Z* scores (see Table S1 in the supplemental material). Figure 2 shows that the proportion of positive sera among the 32 infected dogs correlated with the  $\log_{10}$  fold difference between each infected dog serum value and the mean of the negative-control samples. Only a few *B. burgdorferi* proteins in the array were not bound by antibodies from the infected dogs in this study. Eighteen of the 69 proteins had lower 95% confidence limits for the *Z* score of  $< 1$ , but most of those proteins had fold differences over control values of  $> 5$ . By a combination of the rankings according to fold differences and frequencies of positive results, the 25 standout ORFs in this study were BBA64, BBA25, BBK32, BBH13, BBA57, BBA36, BB0147, BBH06, BBN38, BB0844, BBB14, BBF41, BBA66, BBL39, BBK19, BBR42, BBM34, BBP34, BBS41, BB0359, BBA04, BB0279, BBN39, BBM27, and BB0518.

For BBA25, decorin-binding protein B, which was bound by sera from all infected dogs and was the top-ranked antigen in the study of human LB sera (23), there was no discernible difference in the distributions of values according to whether the dog had received the experimental vaccine in the past; the mean pixel intensities per spot of antibody binding for all proteins in the array were 10,692 pixels per spot (95% CI, 7,958 to 14,365 pixels per spot) for the vaccinated group and 6,981 pixels per spot (95% CI, 3,115 to 15,647 pixels per spot) for the unvaccinated group ( $t$  test  $P = 0.23$ ; Mann-Whitney  $P = 0.48$ ). Highly prevalent immunogens in this array also included the flagellar structural proteins FlaB (BB0147), FliL (BB0279), and FlgE (BB0283) and the flagellar biosynthesis protein FliZ (BB0276). Other chromosome-encoded proteins frequently bound by antibodies in both infected dog sera and human LB sera (23) were the chaperone DnaK (BB0518), a carboxy-ter-



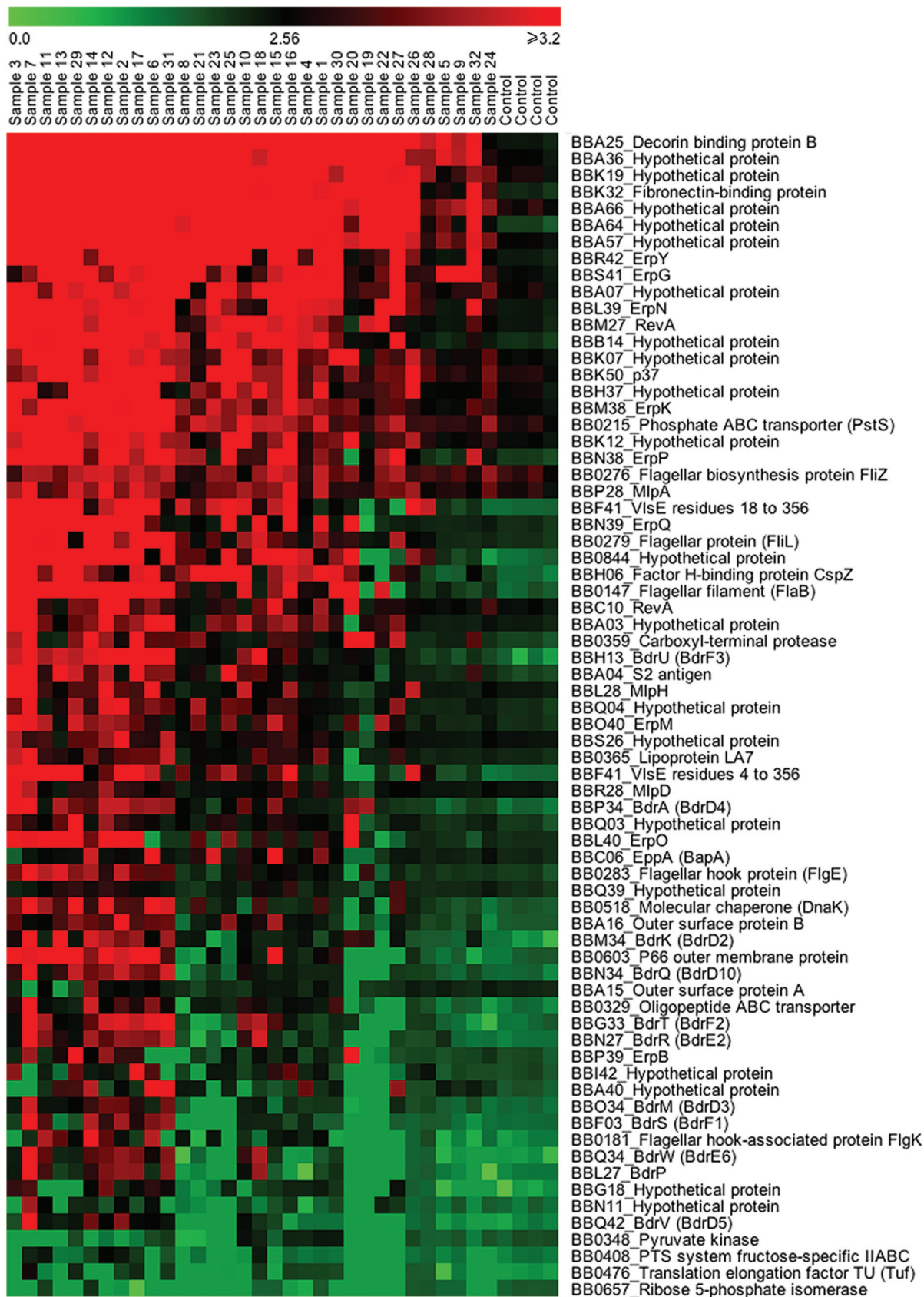
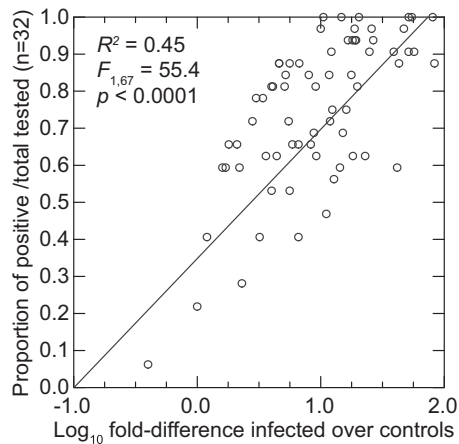


FIG 1 Two-color display of an analysis of intensity of antibody binding to 69 proteins of *B. burgdorferi* by sera from infected and control dogs. The gradient heatmap depicts the  $\log_{10}$ -transformed signal intensity values of serum antibody binding to *B. burgdorferi* proteins; gradient colors indicate the range of binding intensity values from 0 (green) to  $\geq 3.2$  (red), with the median value of 2.56 in black. Individual dog serum samples are arranged by the total sums of pixel values for binding to the proteins in the array, while proteins are listed by averaged intensities of responses among infected serum samples. Where known, the gene name for the ORF of each protein is shown.



**FIG 2** Scatter plot of antibody binding to proteins printed as *in vitro* transcription-translation reactions, for 69 recombinant proteins of *B. burgdorferi* reacted with sera from 32 beagle dogs infected with *B. burgdorferi* and 4 uninfected dogs. The proportion of positive sera for each of the 69 proteins is plotted against the  $\log_{10}$  fold difference of the mean of the infected sera over the mean of the control sera. The  $F$  statistic and coefficient of determination ( $R^2$ ) for the linear regression are shown.

minal protease (BB0359), lipoprotein LA7 (BB0365), and the phosphate ABC transporter (BB0215).

**Plasmid-encoded proteins.** Several other proteins encoded by the linear plasmid lp54 besides BBA25 were detectably immunogenic for the infected dogs, including BBA64, BBA36, and BBA57. The reactivities of sera from infected dogs against BBA15 (the OspA protein and the basis of the experimental vaccine) were not distinguishable from those of uninfected controls by any criterion (see Table S1 in the supplemental material). There was no discernible difference in the distributions of OspA binding values for the 23 infected dogs that had received an experimental vaccine formulation at least 37 weeks earlier and the 9 dogs that had not, i.e., 799 pixels per spot (95% CI, 677 to 944 pixels per spot) and 733 pixels per spot (95% CI, 589 to 911 pixels per spot), respectively ( $t$  test  $P = 0.57$ ; Mann-Whitney  $P = 0.67$ ). Mean binding values for BBA16 (OspB, the other protein encoded by the *ospAB* operon [37]) were severalfold higher than control values for this protein, but the latter values were exceptionally low, and the differences between infected and control sera were not significant at the 0.05 level. The 5 represented lipoproteins encoded by plasmid lp36 were BBK07, BBK12, BBK19, BBK32, and BBK50, and all were highly immunogenic in these infections. The paralogs BBK07 and BBK12 are 87% identical in amino acid sequences (23) and, not unexpectedly, there was a strong correlation (correlation coefficient, 0.96) between the reactivities to these proteins among the sera.

Several of the highest ranking proteins in the array were members of the PF80 family, i.e., the plasmid-encoded *Borrelia* direct repeat (Bdr) proteins. The high-ranking group included BdrU, BdrA, and BdrK. Among the less-reactive PF80 members was BdrT (BBG33), a placement that contrasted with the comparatively greater reactivity of BdrT with human sera (23). Correlations of antibody binding signals for all members of the PF80 family ranged from 0.57 for BBN27 and BBP34 to 0.98 for BBL27 and BBQ34. The other set of paralogous proteins that had multiple representatives in the array was the Erp proteins. Members of

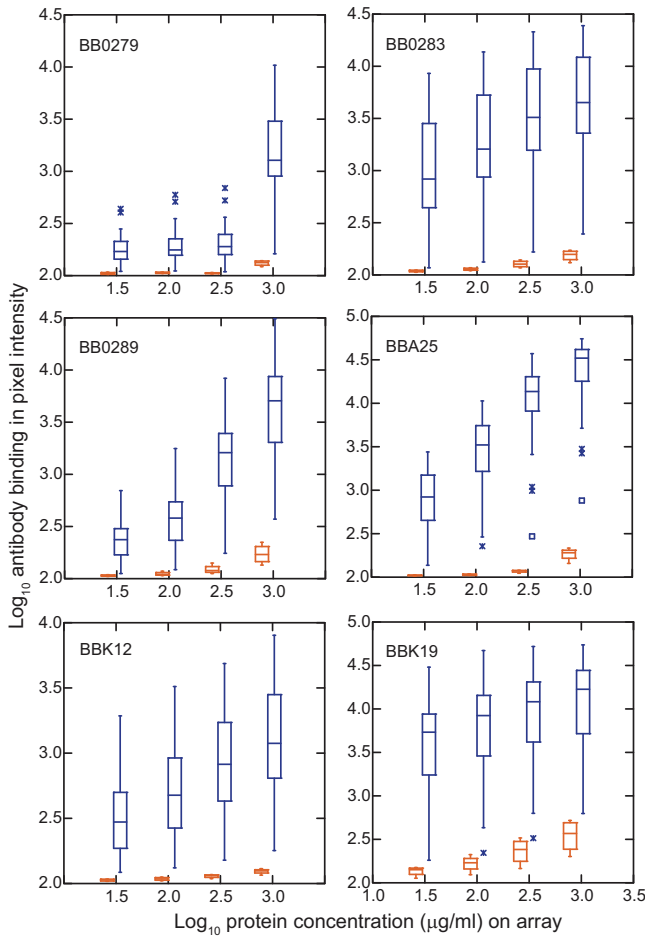
the three subfamilies of Erp proteins, i.e., PF162, PF163, and PF164, were included. With the exception of ErpB (BBP39), all Erp proteins either elicited antibodies during infection or were sufficiently cross-reactive with another Erp protein that was expressed. The correlations between the different members ranged from a low of 0.45 for the ErpG-ErpM pair to a high of 0.76 for the ErpK-ErpY pair.

Among the immunogenic proteins were two presumptive lipoproteins that have not been well characterized, namely, chromosome-encoded BB0844 and plasmid-encoded BBB14; these proteins were bound by antibodies in 28 (88%) and 31 (94%) sera, respectively, from the 32 infected dogs (see Table S1 in the supplemental material). Although the *bb0844* gene is located at the right end of the linear chromosome in some strains (38, 39), it is a member of the PF12 family and is homologous to several plasmid-encoded proteins, such as BBH37.

**Antibody binding to proteins at different concentrations.** To assess the effects of the amounts of antigens in the array, we examined the binding of serum antibodies to recombinant proteins that had been purified and printed in the array in various amounts over a 30-fold range. Figure 3 shows binding intensities plotted against the different concentrations for sera from infected and control animals for the recombinant proteins FliL (BB0279), FlgE (BB0283), FliH (BB0289), DbpB (BBA25), BBK12, and BBK19. In the case of BBK19, the greatest differences in binding values between sera from infected and uninfected dogs were in the spots with the lowest protein concentrations; for the other proteins, the differences in reactivities between the infected sera and the control sera were significant at all concentrations studied.

**Antibodies to OspC proteins.** Sera from infected dogs bound to all or most of the OspC proteins in the second array. The mean pixel intensities of antibody binding to all OspC proteins were 1,808 pixels per spot (95% CI, 1,161 to 2,817 pixels per spot) for 32 infected dog sera and 5 pixels per spot (95% CI, 2 to 11 pixels per spot) for 4 uninfected dog sera ( $P < 0.0001$ ). The mean pixel intensities of antibody binding to all OspC proteins in the array were 1,657 pixels per spot (95% CI, 919 to 2,918 pixels per spot) for the 23 dogs that had received an experimental vaccine and 2,259 pixels per spot (95% CI, 1,381 to 3,694 pixels per spot) for the 9 dogs that had not ( $t$  test  $P = 0.55$ ; Mann-Whitney  $P = 0.43$ ). Individual sera from the infected dogs had antibodies to a mean of 16 and a median of 18 (range, 2 to 24) of the 24 OspC proteins. For each of the 24 proteins, a mean of 22 and a median of 23 (range, 7 to 31) of the 32 sera were positive. With the exception of types C, G, T, and U, OspC proteins represented in the array were each bound by antibodies from the majority of sera; these included types (such as H3 and L) that would be unlikely to be present in ticks from the northeastern United States, on the basis of what is known of their geographic distributions (36). Figure S1 in the supplemental material gives the distributions of reactivities for each dog serum and for each OspC.

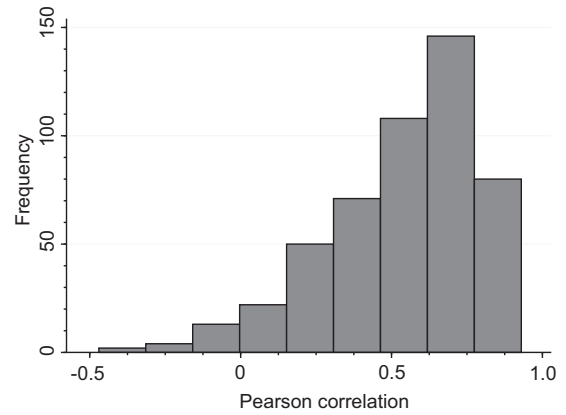
**Differences between dogs in antibody responses.** Given that 25 adult field-collected ticks, with an estimated infection prevalence of  $\sim 50\%$ , were used to initiate infections, it was likely that most of the dogs not only became infected with *B. burgdorferi* but also became infected with  $\geq 2$  strains each, as expected for natural reservoirs such as *Peromyscus leucopus* (40). It was not surprising that most of the sera had antibodies that bound to the majority of the OspC proteins, as the result of either mixed infections or antigenic cross-reactivity between OspC pairs (25). The latter phe-



**FIG 3** Binding of antibodies in dog sera to purified proteins of *B. burgdorferi* at different concentrations. Box-whisker plots show the  $\log_{10}$ -transformed intensity values for antibody binding of 32 infected (blue) or 4 uninfected (red) dog serum samples to purified recombinant proteins encoded by ORFs BB0279 (FliL), BB0283 (FlgE), BB0289 (FliH), BBA25 (DbpB), BBG33, BBK12, and BBK19, at concentrations of 30, 100, 300, or 900  $\mu\text{g/ml}$ . Each box indicates the first and third quartiles, and the line inside the box is the median. The  $1.5\times$  interquartile range is indicated by the vertical line bisecting the box, and values outside this range are indicated by asterisks.

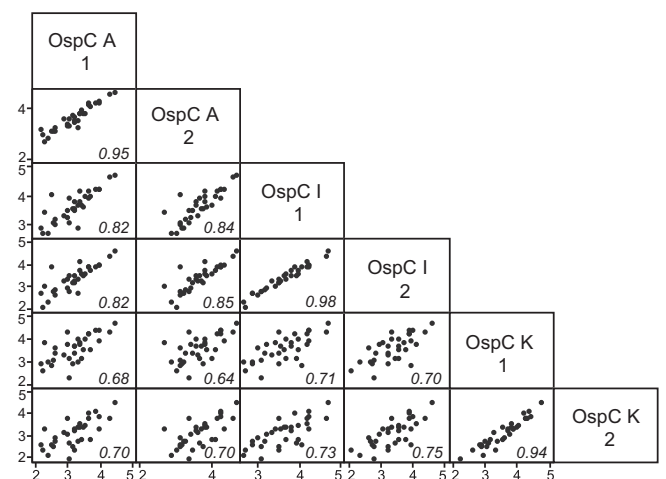
nomenon may account for binding to types restricted to the mid-western or western United States (36, 41). However, there was also evidence that diversity in responses among individual dogs might not be wholly attributable to infections with different strains. Heterogeneity of anti-OspC responses was also demonstrated by the frequency distribution of 496 pairwise correlations of the log-transformed antibody binding values for all 32 sera against each of 24 OspC types, with exclusion of the homotypic pairs (Fig. 4; see also Table S2 in the supplemental material). The mean of the leftward-skewed distribution was 0.53, with 5th percentile and 95th percentile values of 0.07 and 0.84, respectively. The heterogeneity and shape of the frequency distribution were comparable to those observed for pairwise correlations with human LB sera (25).

We further investigated the specificity of anti-OspC antibodies for three OspC proteins, i.e., A, I, and K, each of which was provided in nonlipidated recombinant form from two different



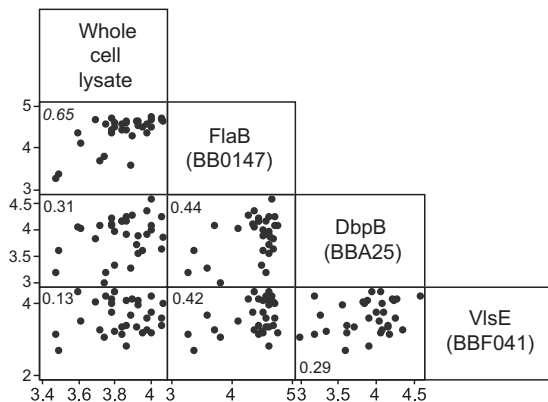
**FIG 4** Frequency distribution of pairwise Pearson correlations for binding of antibodies in 32 sera from *B. burgdorferi*-infected dogs against 24 OspC types. The values for each of the 496 pairwise correlations are given in Table S2 in the supplemental material.

sources, namely, our laboratory and a commercial source that carried out expression and purification of the His-tagged recombinant proteins. Types A, I, and K are commonly represented in the *B. burgdorferi* population in the northeastern United States (26). If a major part of the observed heterogeneity of responses was attributable to interpad variations during printing or antibody processing, then we would expect that correlations between OspC proteins of the same type but from different sources would be similar in degree to the correlations between types, regardless of the source; however, this was not the finding. As shown in Fig. 5, the strongest correlations were between the homotypic pairs of types A, I, and K from different sources. There were also significant correlations between the pairs of different types, no matter the source, but, as the plots reveal, individual sera had different profiles of responses to the three OspC types, as was found with human LB sera (25).



**FIG 5** Matrix of scatter plots of  $\log_{10}$ -transformed values for binding of antibodies in sera from 32 *B. burgdorferi*-infected dogs to 3 different OspC proteins (A, I, and K) from two sources each. Source 1 was the University of California, Irvine, laboratory, and source 2 was a company providing recombinant protein expression and purification (see text). The  $x$  and  $y$  axes present  $\log_{10}$  values. The Pearson correlations for each pair are shown in the cells. Correlations with  $P$  values of  $<0.01$  are shown in italics.





**FIG 6** Matrix of scatter plots of  $\log_{10}$ -transformed values for binding of serum antibodies from 32 *B. burgdorferi*-infected dogs to a whole-cell lysate of *B. burgdorferi* and to the purified recombinant proteins FlaB, DbpB, and VlsE. The x and y axes present  $\log_{10}$  values. The Pearson correlations for each pair are shown in the cells. Correlations with *P* values of  $<0.01$  are shown in italics.

This analysis was then extended to other antigens in purified form in the second array, i.e., FlaB, Flil, FlgE, P39, P83, DbpB, and VlsE, as well as the whole-cell lysate. In particular, we were interested in identifying covariates of the antibody responses, across all sera, to the array's spots of whole-cell lysate, which was a proxy for whole-cell-based assays like ELISAs. The plots and correlations for the lysate, FlaB, DbpB, and VlsE are shown in Fig. 6; these and the other plots are in Fig. S2 in the supplemental material. By this analysis, as well as a separate analysis that included BBK07, BBK12, and BBK19 (data not shown), the strongest correlation with the lysate results was for the values for binding to FlaB.

This was confirmed by a generalized linear model with stepwise and backward exclusion of independent variables. Antibody binding to FlaB was the best single predictor of lysate reactivity for all dogs ( $R^2 = 0.43$ ,  $P < 0.001$ ). Although both VlsE and DbpB had high prevalences of reactivity and net values among the dogs (see Table S1 in the supplemental material), neither DbpB ( $R^2 = 0.10$ ,  $P = 0.08$ ) nor VlsE ( $R^2 = 0.02$ ,  $P = 0.46$ ) was an informative predictor of the corresponding whole-cell lysate result for a given serum sample. The discordance of the values for immunodominant VlsE or DbpB with FlaB, as well as with other flagellar antigens such as FlgE, was further evidence of individual differences among these beagles in their responses to infection.

## DISCUSSION

There were two principal findings of the study. The first was that these domestic dogs, which had been infected with *B. burgdorferi* experimentally through tick transmission, developed antibody responses that were qualitatively similar to those of humans with LB and naturally infected *P. leucopus* in their profiles of reactivity on an array (23). There were differences as well, and these are discussed. However, the overarching conclusion is that a set of the immunodominant antigens for humans, including DbpB, VlsE, FlaB, Flil, FlgE, DnaK, BBK19, BBK32, BBA36, BBA57, BBA64, lipoprotein LA7, and several Bdr and Erp proteins, largely corresponded to the highly prevalent immunogens for the infected dogs.

Although this was the broadest attempt to date to identify important antigens in active *B. burgdorferi* infections in dogs, we do not rule out the possibility that ORFs not included in the array

may be informative antigens in canine infections. For such an assessment, a full genome-wide proteome should be used. Another limitation of the study was the small number of controls. The sample size was sufficient for the identification of immunogenic proteins, but we did not attempt more than a rough ranking of these proteins. For further development of immunoassays, a similar array study should be carried out with sera from dogs of various breeds that have been naturally infected and the results compared with those for sera from a number of seronegative dogs in the area in which the disease is endemic, as well as dogs located in areas without *B. burgdorferi* transmission. However, the study revealed features of the host responses that would be more difficult to discern with a larger but more heterogeneous group of subjects and circumstances of infection.

Two studies reported an  $\sim 20$ -kDa band on immunoblots of whole-cell lysates that was commonly bound by antibodies in sera from infected beagles (30, 31). What those investigators identified in the blots may have been DbpB (BBA25) or its more-polymorphic operon-mate DbpA (BBA24), with sizes of 19 to 20 kDa each. Another candidate is BBB14, of  $\sim 19$  kDa. BBB14 in the array was frequently bound by antibodies from infected canines in the present study but was recognized seldom by humans and not at all by white-footed mice (23). A predicted lipoprotein, BBB14 is not a member of a known paralogous family and is unlike any other protein in the database. We infer that BBB14 was expressed during canine infections and elicited an antibody response. Consequently, it may be an informative and specific serological marker for canine infections.

Binding of antibodies to the whole-cell lysate in the array significantly covaried with binding of antibodies to the major structural flagellin protein FlaB and to a lesser extent with binding of those to other constitutively expressed structural proteins, such as the flagellar protein Flil and the protoplasmic cylinder-associated protein P83, orthologs of which are found in treponemes and leptospire. In contrast, there was little correlation between the binding values for the lysate and those for VlsE and DbpB, which otherwise were commonly recognized proteins both in this study and in a previous study of human and *P. leucopus* sera (23). Neither VlsE nor DbpB would be expected to be expressed highly, if at all, under the conditions of *in vitro* growth used for *B. burgdorferi* here, and consequently they would likely be absent or present at low concentrations in the whole-cell lysate (42). It is conceivable that a "cocktail" of a few recombinant proteins that included an antigen (or two), such as FlaB or Flil, that strongly correlates with assays based on whole cells, together with an antigen, such as DbpB or VlsE, that is expressed more highly *in vivo* than *in vitro*, would provide both sensitivity and specificity for immunoassays for canine antibodies to LB *Borrelia* spp.

As described and discussed in part above, all of the dogs had antibodies to multiple OspC types. This may be attributable to the three factors in play here, i.e., (i) infection with more than one strain, (ii) cross-reactivity between OspC proteins at the N- and C-terminal regions (24, 25, 43), and (iii) decreasing specificity of the anti-OspC response as the infection progresses and a larger number of epitopes, some of which are conserved, elicit responses (44, 45). The sera were from dogs that had been infected for at least 11 weeks after placement of 25 ticks, about one-half of which were probably bearing *B. burgdorferi* of one type or another. We observed levels of antibody binding to OspC proteins that were similar for OspC types that are typical of the region where the infected



ticks originated (e.g., types K, A, B, N, U, H, F, and I) and types that are either absent (types A3, C3, D3, E3, F3, H3, and I3) or rare (types B3, L, and J) in that *B. burgdorferi* population (26).

The second main finding was the demonstration of the diversity of antibody responses of individual animals to the antigens in the arrays under these experimental conditions. The focus shifts to differences among infected individuals, as opposed to comparisons between the infected and uninfected groups. These differences in the breadth and amount of anti-*B. burgdorferi* antibodies among dogs are illustrated in Fig. 1. However, this represented only one part of that response, i.e., antibodies elicited by the infection process. Other elements of the host response, such as innate immunity and the cellular arm of adaptive immunity, were not examined. While there were compelling reasons for using ticks rather than needle inoculations for infection and for applying a sufficient number of ticks to ensure infection, it is possible that some dogs also acquired from the field-collected ticks unapparent infections with *A. phagocytophilum*, *B. microti*, *B. miyamotoi*, a *Bartonella* sp., or the deer tick virus, and in such cases the antibody responses to the coinfecting *B. burgdorferi* might have been affected. There also might have been differences between dogs in the total inocula of *B. burgdorferi* cells that were transmitted to them, but we doubt that this was a large range. For a sample size of 25 ticks and an expected probability of tick infection of 0.5, the 95% confidence interval for the number of infected ticks to feed on an individual dog would be 8 to 18 with a binomial distribution model.

The study was restricted to a single breed and the same experimental conditions for infection. The serum samples were obtained 11 to 12 weeks following tick placement, which represented sufficient time for infection and for IgG responses to infection to occur. All of the dogs had been vaccinated with an experimental formulation of an OspA vaccine 37 or 66 weeks prior to challenge but were seronegative for antibodies to OspA at that point. If the immunization protocol were insufficient to elicit detectable specific antibody responses, then it is unlikely that an unspecified effect of this lipoprotein would have lasted for so many months. Although the major histocompatibility complex (MHC) class I and II genotypes of the dogs in the study are not known, it is likely that, in comparison with the population of all dog breeds, there were relatively few MHC haplotypes represented among the beagles (46). This means that the range of potential antibody responses might have been restricted but also that the differences in traits that were observed may eventually be more feasibly associated with a genetic locus in beagles than would be the case for a population with a different demographic history, a larger effective size, and less linkage disequilibrium (34).

On the basis of these results, we propose that there are at minimum two phenotypes of host responses to *B. burgdorferi* that might be exploited for mapping of quantitative trait loci by GWAS or targeting of MHC loci. The first phenotype is an antibody response to a relatively conserved and ubiquitous protein, such as DbpB, that is immunogenic for most (if not all) animals but elicits different amounts and/or qualities of antibodies among animals and shows weak correlation with reactivities against other highly immunogenic proteins, such as FlaB (Fig. 5). The second phenotype involves the specificity of antibodies to OspC proteins. Even under conditions where antibodies to multiple types were expected and observed, from either mixed infections or shared epitopes, the dogs differed in their patterns of reactivities against

the battery of OspC types (Fig. 4; see also Fig. S1 in the supplemental material). Antibodies to the various OspC types and differences between dogs in the specificities of their antibodies also have implications for the development of OspC-based vaccines against *B. burgdorferi* (47).

## ACKNOWLEDGMENTS

This work was supported in part by NIH grants AI078734 (to E.B.), AI065359 (to A.G.B.), and AI100236 (to A.G.B.).

We thank Yung-Fu Chang at Cornell University for providing bacterial isolates and Bridgit Travinsky and Jonas Bunikis for curating the isolates.

## REFERENCES

- Little SE, Heise SR, Blagburn BL, Callister SM, Mead PS. 2010. Lyme borreliosis in dogs and humans in the USA. *Trends Parasitol.* 26:213–218. <http://dx.doi.org/10.1016/j.pt.2010.01.006>.
- Hinrichsen VL, Whitworth UG, Breitschwerdt EB, Hegarty BC, Mather TN. 2001. Assessing the association between the geographic distribution of deer ticks and seropositivity rates to various tick-transmitted disease organisms in dogs. *J. Am. Vet. Med. Assoc.* 218:1092–1097. <http://dx.doi.org/10.2460/javma.2001.218.1092>.
- Bowman D, Little SE, Lorentzen L, Shields J, Sullivan MP, Carlin EP. 2009. Prevalence and geographic distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey. *Vet. Parasitol.* 160:138–148. <http://dx.doi.org/10.1016/j.vetpar.2008.10.093>.
- Johnson JL, Ginsberg HS, Zhioua E, Whitworth UG, Jr, Markowski D, Hyland KE, Hu R. 2004. Passive tick surveillance, dog seropositivity, and incidence of human Lyme disease. *Vector Borne Zoonotic Dis.* 4:137–142. <http://dx.doi.org/10.1089/1530366041210710>.
- Guerra MA, Walker ED, Kitron U. 2001. Canine surveillance system for Lyme borreliosis in Wisconsin and northern Illinois: geographic distribution and risk factor analysis. *Am. J. Trop. Med. Hyg.* 65:546–552.
- Duncan AW, Correa MT, Levine JF, Breitschwerdt EB. 2005. The dog as a sentinel for human infection: prevalence of *Borrelia burgdorferi* C6 antibodies in dogs from southeastern and mid-Atlantic states. *Vector Borne Zoonotic Dis.* 5:101–109. <http://dx.doi.org/10.1089/vbz.2005.5.101>.
- Greene RT. 1990. An update on the serodiagnosis of canine Lyme borreliosis. *J. Vet. Intern. Med.* 4:167–171. <http://dx.doi.org/10.1111/j.1939-1676.1990.tb00891.x>.
- Chang YF, Novosel V, Chang CF, Summers BA, Ma DP, Chiang YW, Acree WM, Chu HJ, Shin S, Lein DH. 2001. Experimental induction of chronic borreliosis in adult dogs exposed to *Borrelia burgdorferi*-infected ticks and treated with dexamethasone. *Am. J. Vet. Res.* 62:1104–1112. <http://dx.doi.org/10.2460/ajvr.2001.62.1104>.
- Summers BA, Straubinger AF, Jacobson RH, Chang YF, Appel MJ, Straubinger RK. 2005. Histopathological studies of experimental Lyme disease in the dog. *J. Comp. Pathol.* 133:1–13. <http://dx.doi.org/10.1016/j.jcpa.2004.11.006>.
- Krimer PM, Miller AD, Li Q, Grosenbaugh DA, Susta L, Schatzberg SJ. 2011. Molecular and pathological investigations of the central nervous system in *Borrelia burgdorferi*-infected dogs. *J. Vet. Diagn. Invest.* 23:757–763. <http://dx.doi.org/10.1177/1040638711408281>.
- Appel MJ, Allan S, Jacobson RH, Lauderdale TL, Chang YF, Shin SJ, Thomford JW, Todhunter RJ, Summers BA. 1993. Experimental Lyme disease in dogs produces arthritis and persistent infection. *J. Infect. Dis.* 167:651–664. <http://dx.doi.org/10.1093/infdis/167.3.651>.
- Callister SM, Jobe DA, Schell RF, Lovrich SD, Onheiber KL, Korshus JB. 2000. Detection of borreliacidal antibodies in dogs after challenge with *Borrelia burgdorferi*-infected *Ixodes scapularis* ticks. *J. Clin. Microbiol.* 38:3670–3674.
- Greene RT, Walker RL, Nicholson WL, Heidner HW, Levine JF, Burgess EC, Wyand M, Breitschwerdt EB, Berkhoff HA. 1988. Immunoblot analysis of immunoglobulin G response to the Lyme disease agent (*Borrelia burgdorferi*) in experimentally and naturally exposed dogs. *J. Clin. Microbiol.* 26:648–653.
- Littman MP, Goldstein RE, Labato MA, Lappin MR, Moore GE. 2006. ACVIM small animal consensus statement on Lyme disease in dogs: diag-

- nosis, treatment, and prevention. *J. Vet. Intern. Med.* 20:422–434. <http://dx.doi.org/10.1111/j.1939-1676.2006.tb02880.x>.
15. Speck S, Reiner B, Streich WJ, Reusch C, Wittenbrink MM. 2007. Canine borreliosis: a laboratory diagnostic trial. *Vet. Microbiol.* 120:132–141. <http://dx.doi.org/10.1016/j.vetmic.2006.10.017>.
  16. Zhang JR, Hardham JM, Barbour AG, Norris SJ. 1997. Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. *Cell* 89:275–285. [http://dx.doi.org/10.1016/S0092-8674\(00\)80206-8](http://dx.doi.org/10.1016/S0092-8674(00)80206-8).
  17. Krause PJ, Narasimhan S, Wormser GP, Barbour AG, Platonov AE, Brancato J, Lepore T, Dardick M, Rollend L, Steeves TK, Diuk-Wasser M, Usmani-Brown S, Williamson P, Sarksyan DS, Fikrig E, Fish D. 2014. Seroreactivity and seroprevalence of *Borrelia miyamotoi sensu lato* infection in the northeastern United States. *Emerg. Infect. Dis.*, in press.
  18. Liang FT, Jacobson RH, Straubinger RK, Grooters A, Philipp MT. 2000. Characterization of a *Borrelia burgdorferi* VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* 38:4160–4166.
  19. Magnarelli LA, Levy SA, Ijdo JW, Wu C, Padula SJ, Fikrig E. 2001. Reactivity of dog sera to whole-cell or recombinant antigens of *Borrelia burgdorferi* by ELISA and immunoblot analysis. *J. Med. Microbiol.* 50:889–895.
  20. Magnarelli LA, Flavell RA, Padula SJ, Anderson JF, Fikrig E. 1997. Serologic diagnosis of canine and equine borreliosis: use of recombinant antigens in enzyme-linked immunosorbent assays. *J. Clin. Microbiol.* 35:169–173.
  21. Barthold SW, Levy SA, Fikrig E, Bockenstedt LK, Smith AL. 1995. Serologic responses of dogs naturally exposed to or vaccinated against *Borrelia burgdorferi* infection. *J. Am. Vet. Med. Assoc.* 207:1435–1440.
  22. Wagner B, Freer H, Rollins A, Erb HN. 2011. A fluorescent bead-based multiplex assay for the simultaneous detection of antibodies to *B. burgdorferi* outer surface proteins in canine serum. *Vet. Immunol. Immunopathol.* 140:190–198. <http://dx.doi.org/10.1016/j.vetimm.2010.12.003>.
  23. Barbour AG, Jasinskas A, Kayala MA, Davies DH, Steere AC, Baldi P, Felgner PL. 2008. A genome-wide proteome array reveals a limited set of immunogens in natural infections of humans and white-footed mice with *Borrelia burgdorferi*. *Infect. Immun.* 76:3374–3389. <http://dx.doi.org/10.1128/IAI.00048-08>.
  24. Baum E, Hue F, Barbour A. 2012. Experimental infections of the reservoir species *Peromyscus leucopus* with diverse strains of *Borrelia burgdorferi*, a Lyme disease agent. *mBio* 3:e00434–00412. <http://dx.doi.org/10.1128/mBio.00434-12>.
  25. Baum E, Randall AZ, Zeller M, Barbour AG. 2013. Inferring epitopes of a polymorphic antigen amidst broadly cross-reactive antibodies using protein microarrays: a study of OspC proteins of *Borrelia burgdorferi*. *PLoS One* 8:e67445. <http://dx.doi.org/10.1371/journal.pone.0067445>.
  26. Barbour AG, Travinsky B. 2010. Evolution and distribution of the ospC gene, a transferable serotype determinant of *Borrelia burgdorferi*. *mBio* 1:e00153–10. <http://dx.doi.org/10.1128/mBio.00153-10>.
  27. Akey JM, Ruhe AL, Akey DT, Wong AK, Connelly CF, Madeoy J, Nicholas TJ, Neff MW. 2010. Tracking footprints of artificial selection in the dog genome. *Proc. Natl. Acad. Sci. U. S. A.* 107:1160–1165. doi:<http://dx.doi.org/10.1073/pnas.0909918107>.
  28. Parker HG. 2012. Genomic analyses of modern dog breeds. *Mamm. Genome* 23:19–27. <http://dx.doi.org/10.1007/s00335-011-9387-6>.
  29. O'Connor TP, Esty KJ, Hanscom JL, Shields P, Philipp MT. 2004. Dogs vaccinated with common Lyme disease vaccines do not respond to IR6, the conserved immunodominant region of the VlsE surface protein of *Borrelia burgdorferi*. *Clin. Diagn. Lab. Immunol.* 11:458–462. <http://dx.doi.org/10.1128/CDLI.11.3.458-462.2004>.
  30. Lovrich SD, La Fleur RL, Jobe DA, Johnson JC, Asp KE, Schell RF, Callister SM. 2007. Borreliacidal OspC antibody response of canines with Lyme disease differs significantly from that of humans with Lyme disease. *Clin. Vaccine Immunol.* 14:635–637. <http://dx.doi.org/10.1128/CDLI.11.3.458-462.2004>.
  31. LaFleur RL, Dant JC, Wasmoe TL, Callister SM, Jobe DA, Lovrich SD, Warner TF, Abdelmagid O, Schell RF. 2009. Bacterin that induces anti-OspA and anti-OspC borreliacidal antibodies provides a high level of protection against canine Lyme disease. *Clin. Vaccine Immunol.* 16:253–259. <http://dx.doi.org/10.1128/CDLI.11.3.458-462.2004>.
  32. Vamathevan JJ, Hall MD, Hasan S, Woollard PM, Xu M, Yang Y, Li X, Wang X, Kenny S, Brown JR, Huxley-Jones J, Lyon J, Haselden J, Min J, Sansseau P. 2013. Minipig and beagle animal model genomes aid species selection in pharmaceutical discovery and development. *Toxicol. Appl. Pharmacol.* 270:149–157. <http://dx.doi.org/10.1016/j.taap.2013.04.007>.
  33. Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, Chang JL, Kulbokas EJ, III, Zody MC, Mauceli E, Xie X, Breen M, Wayne RK, Ostrander EA, Ponting CP, Galibert F, Smith DR, DeJong PJ, Kirkness E, Alvarez P, Biagi T, Brockman W, Butler J, Chin CW, Cook A, Cuff J, Daly MJ, DeCaprio D, Gnerre S, Grabherr M, Kellis M, Kleber M, Bardeleben C, Goodstadt L, Heger A, Hitte C, Kim L, Koepfli KP, Parker HG, Pollinger JP, Searle SM, Sutter NB, Thomas R, Webber C, Baldwin J, Abebe A, Abouelleil A, Aftuck L, Ait-Zahra M, et al. 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* 438:803–819. <http://dx.doi.org/10.1038/nature04338>.
  34. Parker HG, Shearin AL, Ostrander EA. 2010. Man's best friend becomes biology's best in show: genome analyses in the domestic dog. *Annu. Rev. Genet.* 44:309–336. <http://dx.doi.org/10.1146/annurev-genet-102808-115200>.
  35. Mather TN, Fish D, Coughlin RT. 1994. Competence of dogs as reservoirs for Lyme disease spirochetes (*Borrelia burgdorferi*). *J. Am. Vet. Med. Assoc.* 205:186–188.
  36. Travinsky B, Bunikis J, Barbour AG. 2010. Geographic differences in genetic locus linkages for *Borrelia burgdorferi*. *Emerg. Infect. Dis.* 16:1147–1150. <http://dx.doi.org/10.3201/eid1607.091452>.
  37. Howe TR, Mayer LW, Barbour AG. 1985. A single recombinant plasmid expressing two major outer surface proteins of the Lyme disease spirochete. *Science* 227:645–646. <http://dx.doi.org/10.1126/science.3969554>.
  38. Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK, Gwinn M, Dougherty B, Tomb JF, Fleischmann RD, Richardson D, Peterson J, Kerlavage AR, Quackenbush J, Salzberg S, Hanson M, van Vugt R, Palmer N, Adams MD, Gocayne J, Weidman J, Utterback T, Watthey L, McDonald L, Artiach P, Bowman C, Garland S, Fujii C, Cotton MD, Horst K, Roberts K, Hatch B, Smith HO, Venter JC. 1997. Genomic sequence of a Lyme disease spirochete, *Borrelia burgdorferi*. *Nature* 390:580–586. <http://dx.doi.org/10.1038/37551>.
  39. Casjens S, Palmer N, van Vugt R, Huang WM, Stevenson B, Rosa P, Lathigra R, Sutton G, Peterson J, Dodson RJ, Haft D, Hickey E, Gwinn M, White O, Fraser CM. 2000. A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*. *Mol. Microbiol.* 35:490–516. <http://dx.doi.org/10.1046/j.1365-2958.2000.01698.x>.
  40. Bunikis J, Tsao J, Luke CJ, Luna MG, Fish D, Barbour AG. 2004. *Borrelia burgdorferi* infection in a natural population of *Peromyscus leucopus* mice: a longitudinal study in an area where Lyme borreliosis is highly endemic. *J. Infect. Dis.* 189:1515–1523. <http://dx.doi.org/10.1086/382594>.
  41. Girard YA, Travinsky B, Schotthoefer A, Fedorova N, Eisen RJ, Eisen L, Barbour AG, Lane RS. 2009. Population structure of the Lyme borreliosis spirochete *Borrelia burgdorferi* in the western black-legged tick (*Ixodes pacificus*) in northern California. *Appl. Environ. Microbiol.* 75:7243–7252. <http://dx.doi.org/10.1128/AEM.01704-09>.
  42. Skare J, Carroll J, Yang X, Samuels D, Akins D. 2010. Gene regulation, transcriptomics, and proteomics, p 67–102. *In* Samuels DS, Radolf J (ed), *Borrelia: molecular biology, host interaction, and pathogenesis*. Caister Academic Press, Norfolk, United Kingdom.
  43. Mathiesen MJ, Christiansen M, Hansen K, Holm A, Asbrink E, Theisen M. 1998. Peptide-based OspC enzyme-linked immunosorbent assay for serodiagnosis of Lyme borreliosis. *J. Clin. Microbiol.* 36:3474–3479.
  44. Earnhart CG, Buckles EL, Marconi RT. 2007. Development of an OspC-based tetraivalent, recombinant, chimeric vaccinogen that elicits bactericidal antibody against diverse Lyme disease spirochete strains. *Vaccine* 25:466–480. <http://dx.doi.org/10.1016/j.vaccine.2006.07.052>.
  45. Marconi RT, Earnhart CG. 2010. Lyme disease vaccines. *In* Samuels DS, Radolf J (ed), *Borrelia: molecular biology, host interaction, and pathogenesis*. Caister Academic Press, Norfolk, United Kingdom.
  46. Kennedy LJ, Barnes A, Happ GM, Quinnell RJ, Bennett D, Angles JM, Day MJ, Carmichael N, Innes JF, Isherwood D, Carter SD, Thomson W, Ollier WE. 2002. Extensive interbreed, but minimal intrabreed, variation of DLA class II alleles and haplotypes in dogs. *Tissue Antigens* 59:194–204. <http://dx.doi.org/10.1034/j.1399-0039.2002.590303.x>.
  47. Earnhart CG, Marconi RT. 2007. OspC phylogenetic analyses support the feasibility of a broadly protective polyvalent chimeric Lyme disease vaccine. *Clin. Vaccine Immunol.* 14:628–634. <http://dx.doi.org/10.1128/CDLI.11.3.458-462.2004>.