Dynamic Longitudinal Antibody Responses during Borrelia burgdorferi Infection and Antibiotic Treatment of Rhesus Macaques

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Infection with Borrelia burgdorferi elicits robust yet disparate antibody responses in infected individuals. A longitudinal assessment of antibody responses to multiple diagnostic antigens following experimental infection and treatment has not previously been reported. Our goal was to identify a combination of antigens that could indicate infection at all phases of disease and response to antibiotic treatment. Because the rhesus macaque recapitulates the hallmark signs and disease course of human Lyme disease, we examined the specific antibody responses to multiple antigens of B. burgdorferi following infection of macaques. Five macaques infected with strain B31 and 12 macaques infected with strain JD1 were included in the analysis. Approximately half of these animals were treated with antibiotics at 4 to 6 months postinoculation. Antibody responses to several B. burgdorferi recombinant antigens, including OspC, DbpA, BBK32, OspA, and OppA-2, were measured at multiple points throughout infection. We have previously shown a decline in the response to the C6 peptide following antibiotic treatment. Responses to OspA and OspC, however, were variable over time among individuals, irrespective of antibiotic treatment. Not every individual responded to BBK32, but anti-DbpA IgG levels were uniformly high and remained elevated for all animals. All responded to OppA-2, with a decline posttreatment that was slow and incomplete. This is the first demonstration of B. burgdorferi OppA-2 antigenicity in nonhuman primates. The combination of DbpA, OspC, OspA, and OppA-2 with the C6 diagnostic peptide has the potential to detect infection throughout all disease phases.

The etiologic agent of Lyme disease, Borrelia burgdorferi, is transmitted to humans predominantly by ticks of the genus Ixodes, resulting in a disease of protean manifestations due to the organisms’ systemic spread and promiscuous organ tropism. Infected individuals may exhibit a skin rash, arthritis, carditis, extreme fatigue, myalgia, and neurological dysfunction. In 2009, over 30,000 confirmed cases were reported to the Centers for Disease Control and Prevention. Hence, Lyme disease is the most commonly reported vector-borne disease in the United States (http://www.cdc.gov/lyme/stats/chartstables/casesbyyear.html). Treatment with antibiotics is generally effective, especially when they are administered soon after onset (4). As such, early and reliable diagnosis is critical for effective cure of Lyme disease patients.

Laboratory diagnosis of Lyme disease is typically made by the detection of patient serum antibodies specific for B. burgdorferi antigens. Detection of antibodies instead of antigens is necessitated by the absence of detectable spirochetes in the bloodstream once the organism has disseminated. Detection of the bacteria or bacterial antigens from blood or skin biopsy specimen is both invasive and of relatively low sensitivity (2). Antigen can sometimes be detected in urine and cerebrospinal fluid, but these tests are neither reliable nor recommended (45). Two of the most commonly used tests for diagnosis in North America are (i) the two-tier test, which includes an enzyme-linked immunosorbent assay (ELISA) and Western blotting using antigen derived from whole-cell lysates, and (ii) the C6 test, which detects antibodies to a specific peptide within a conserved region of the B. burgdorferi antigen VlsE (5, 30, 32). The C6 test has also been used experimentally for evaluation of treatment efficacy (36, 37).

While the two-tier and C6 tests are suitable for a majority of patients, neither is completely specific or sensitive enough to diagnose all patients. The C6 test, for instance, is more sensitive for human patients with early or late disseminated disease than for patients in the localized phase (5, 16). While the C6 peptide is highly conserved, other ELISA and Western blotting tests utilize whole-cell lysates or recombinant proteins from one species/strain/isolate, despite the enormous potential for antigenic variability that can preclude recognition by antibody. Western blotting will also specifically detect antibodies that recognize the antigen in a fully or partially denatured state, so those antibodies that target conformational epitopes are missed. Furthermore, the potential for patient serum cross-reactivity with antigens shared with other bacteria has led to stringent diagnostic criteria that can confound Western blot interpretation and thus hinder accurate diagnosis (6).

Along with C6 or VlsE, several other B. burgdorferi proteins that are known to elicit antibody responses in natural infections and have been incorporated into immunoblotting-based diagnostics include outer surface protein C (OspC) (44), the fibronectin-binding protein BBK32 (29), decorin-binding protein A (DbpA) (18), flagellar protein, FlaB, and outer surface protein A (OspA). The temporal induction and magnitude of the B cell response to each of these, characterized primarily in mice, are different. OspC, for example, is highly immunogenic, contains antigenic regions that vary among isolates, and is expressed early in infection and then repressed at the advent of humoral immune responses (28).
Antibodies (IgM and IgG) to OspC often appear early in infection (24, 34). DbpA is expressed within the first few days of infection and continues postdissemination, so the antibody response can remain, even in late disease (18). FlAβ is constitutive and immunogenic but holds the potential to be detected by cross-reactive antibodies from other bacterial species, especially when denatured as in Western blotting (17). OspA is expressed when *B. burgdorferi* is in the tick, but its expression is repressed as the spirochetes traverse to the host during tick feeding (40). However, studies indicate that expression of OspA and subsequent antibody responses may return during long-term infections in Lyme arthritis patients (3, 26). As a model for human Lyme disease, *B. burgdorferi*-infected nonhuman primates (NHP) are known to elicit bold and specific antibody responses to multiple antigens (33). Importantly, the responses may differ from those produced by mice (33). We also report here that nonhuman primates develop antibodies to the peptide transporter protein OppA-2. This response appears to decline with antibiotic treatment but more slowly and incompletely than the anti-C6 response (15).

This report presents the first assessment of temporal changes in levels of specific antibodies to multiple antigens following experimental infection with *B. burgdorferi*. By examining the responses in nonhuman primates before and after antibiotic treatment, we empirically determined antigens that would be appropriate for inclusion into a multiplex test to detect infection at multiple phases and as an indicator of treatment outcome.

### MATERIALS AND METHODS

#### Animals, infection, and treatment
Practices in the housing and care of animals conformed to the regulations and standards of the PHS Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals. The Tulane National Primate Research Center is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care-International. The Institutional Animal Care and Use Committee (IACUC) of the Tulane National Primate Research Center approved all animal-related protocols, including the infection, treatment, and sample collection from nonhuman primates. All animal procedures were overseen by veterinarians and staff. For blood collection, monkeys were anesthetized with ketamine (10 mg/kg) by intramuscular injection. The infection and treatment of these animals were described previously (15). The experimental design reflects the assay of antimicrobial efficacy against early disseminated infection (B31-infected macaques) or late disseminated infection (JD1-infected macaques). The repository of serum samples collected over time from these animals afforded the opportunity to assess serological responses to multiple antigens before, during, and after antibiotic treatment.

#### Strain B31-infected animals
Five male rhesus macaques (Chinese origin), 3 to 4 years of age, were given three inoculations in the ventral midline of *in vitro* cultured late log-phase *B. burgdorferi* strain B31, isolated 5A19 (39), as follows: two subcutaneous 1.0-ml injections and one intradermal 0.1-ml injection, each containing 1 × 10^8 organisms diluted in sterile Hanks balanced salt solution (HBSS), for a total inoculum of 3 × 10^8 organisms. At 4 months postinoculation (p.i.), three of the five animals received antibiotic treatment consisting of one 50-mg tablet of doxycycline (Bio-Serv) twice daily for 28 consecutive days. This dose corresponded to >12 mg/kg of body weight/day to ensure that an effective blood level was achieved. Blood was collected at the following time points: 0, 2, 6, 10, 14, 16, 18, 22, 26, 28, 34, 40, and 47 weeks p.i.

#### Strain JD1-infected animals
Briefly, 24 rhesus macaques (Chinese origin) were each given an inoculation of 3.2 × 10^8 spirochetes of the JD1 strain (38) via needle and syringe. Four additional animals were sham inoculated as uninfected controls. At 27 weeks p.i., 12 of the infected animals and 2 of the controls were treated with ceftriaxone, followed by doxycycline. Animals received intravenous ceftriaxone at 25 mg/kg once daily for 30 days, followed by 60 days of oral doxycycline at 2 mg/kg twice daily. Twelve infected and two control animals were sham treated. Blood was collected prior to inoculation and every week for more than a year until the time of euthanasia (54 to 60 weeks p.i.). The serum antibody responses of half of the animals (randomly selected) in each test group were included in our assessment.

### Recombinant protein expression and purification
Each *B. burgdorferi* antigen was produced as a glutathione (GST) fusion protein by inserting the gene of interest into the pGex 4T-1 vector (GE Healthcare). Genes were cloned by primer insertion of BamHI and XhoI restriction enzyme sites for digestion and ligation into the expression vector. The full open reading frames of DbpA and BBK32 were amplified from *B. burgdorferi* strain B31 template DNA using the following primers: 5′-CATCGGATCCATGTTATTTTAAATGGAAT-3′ (DbpA forward), 5′-CATCCTCGAGTTATTTTAAATGGAATGTT-3′ (DbpA reverse), 5′-GACTCGGATCCATGTTATTTTAAATGGAATGTT-3′ (BBK32 forward), and 5′-CATCCTCGAGTTATTTTAAATGGAATGTT-3′ (BBK32 reverse). OspA genes from strains B31 and JD1 were cloned by insertion of BamHI and XhoI restriction enzyme sites so as to omit the leader sequence, beginning with the 20th amino acid of the coding sequence, using the conserved forward primer 5′-CATACTGATGCAATTCAGGGAAAGATGGGAAT-3′ and reverse primers 5′-GACTCGGATCCATGTTATTTTAAATGGAATGTT-3′ (BBK32 reverse). OspA genes from strains B31 and JD1 were cloned by insertion of BamHI and XhoI restriction enzyme sites so as to omit the leader sequence, beginning with the 20th amino acid of the coding sequence, using primers 5′-CATACTGATGCAATTCAGGGAAAGATGGGAAT-3′ (forward) and 5′-CATACTGATGCAATTCAGGGAAAGATGGGAAT-3′ (reverse); the entire oppA2 gene was amplified with primers and cloned into the pGex 4T-1 vector using 5′-GATAGTTATTTTAAATGGAATGTT-3′ (forward) and 5′-GATAGTTATTTTAAATGGAATGTT-3′ (reverse). Plasmids with productive insertions were used to transform Top10 cells and screened. To improve protein expression, BL21 competent cells were transformed with each plasmid. Protein expression was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) for 4 h or overnight. GST fusion proteins were extracted from *Escherichia coli* and purified with a Pierce B-PER Bacterial Protein Extraction Reagent GST Fusion Purification Kit (ThermoScientific). Protein expression and purity were confirmed by Coomassie blue gel staining and immunoblotting using the mouse monoclonal anti-GST antibody clone GST-2 (Sigma Life Science).

### TABLE 1 Summary of monkey groups, antigens tested, and results

| Inoculum and treatment group | Antigen response (no. of positive animals/no. of animals tested) |
|-----------------------------|---------------------------------------------------------------|
| B31 Uninfected              | C6^b 2/2 DbpA 3/3 BBK32 3/3 OspA 3/3 OppA-2 3/3 |
| B31 Treated                 | 3/3 (early); 3/3 (late) |
| JD1 Uninfected              | 12/12 (early); 5/5 (late) |
| JD1 Treated                 | 0/12 ND 5/5 5/5 6/6 |
| JD1 Uninfected              | 0/4 ND 0/1 |
| Total                       | 15/15 3/5 16/16 16/16 17/17 |

a ND, not determined. Bold indicates P < 0.001 by Fisher’s exact test. Values for early and late responses are indicated. See reference 16.

### References

(24, 34) (15) (33) (26) (18) (17) (16) (33).
ELISAs, antibodies, and measurement of endpoint dilution titer (EPDT). Specific IgG and IgM titers were measured and compared. Specificity was measured with preimmune serum and immune serum tested on GST alone purified from bacterial lysate, and none of the sera reacted.

Standard IgG and IgM ELISAs. The ELISAs were performed essentially as described previously (21). Briefly, plates were coated with 0.5 to 1.0 μg/well of recombinant protein. After a blocking step with nonfat milk, serum was added at a dilution of 1:200. Anti-GST antibody (Sigma) was used as a positive control, and peroxidase-labeled goat anti-monkey IgG or goat anti-monkey IgM (KPL, Gaithersburg, MD) secondary antibodies were used at a 1:1,000 dilution. Absolute optical density at 450 nm (OD450) was determined for each sample by subtracting the average value given by preimmune serum from that animal. The number of infected animals that were positive by each test (above the mean value plus 3 standard deviations [SD] for preimmune serum) versus those that were negative was compared by Fisher’s exact test for significance.

RESULTS

Variation among longitudinal IgG responses to multiple antigens. Serum samples from macaques were tested for IgG antibodies against four different proteins (DbpA, BBK32, OspA, and OspC) that have been used in Lyme disease diagnosis by standard ELISA or immunoblotting. The data presented here are from five macaques followed for 11 months of infection; two were untreated, and three were treated with antibiotics. We have supplemented these results with other serum specimens from strain JD1-infected and treated macaques. A summary of the antigen reactivities among these groups can be found in Table 1.

DbpA and BBK32. In mice, DbpA is known to be a T-cell-independent antigen to which a high proportion of the antibody response is directed (9, 42). It has been tested as a vaccine immunogen (11, 20, 21) and as a diagnostic antigen in immunoblotting (45) and ELISA (35). The infected monkeys all produced changes in antibody titer with treatment, the percent change was calculated as follows: (peak titer − endpoint titer)/(peak titer).

FIG 1 Anti-DbpA IgG responses in macaques infected with B. burgdorferi strains B31 (A and B) and JD1 (C and D). Treatment was administered from weeks 16 to 20 for B31-infected monkeys and from weeks 27 to 39 for JD1-infected monkeys. Shown is the average of duplicate or triplicate OD450 readings, with the average value for each individual animal’s preimmune serum response subtracted. The EPDTs shown in panel B were determined for B31-infected macaques at the height of the response (week 22) and at the endpoint of the study (week 47). wk, week.

FIG 2 Anti-BBK32 responses in strain B31-infected macaques. The relative longitudinal responses are shown in panel A, and the reciprocal EPDTs are shown in panel B.
high-titer, sustained responses to DbpA (Fig. 1). The anti-DbpA titers remained elevated in the five animals that were infected with strain B31 spirochetes (Fig. 1A and B), while reactivity was more variable in animals infected with spirochetes of the JD1 strain (Fig. 1C and D). The origin of the recombinant detection antigen (B31) may affect reactivity, but all animals showed a detectable response to DbpA. BBK32 is another extracellular matrix (fibronectin) binding protein that has been tested as both a vaccine (11) and a diagnostic antigen (35). Only three of the five B31-infected macaques produced strong responses to BBK32 though the titer dropped substantially for one of the treated monkeys (Fig. 2). Due to this low responsiveness, we did not test monkeys that were inoculated with JD1 spirochetes for responses to BBK32.

OspA and OspC. For interpretation of the antibody response to OspA, it should be mentioned that these animals were infected by needle inoculation of in vitro cultured spirochetes, which express OspA. Because expression of OspA is downregulated in the feeding tick and remains as such in the mammalian host immediately after transmission, the initial responses are not representative of a natural infection. Thus, all animals responded to OspA, as
predicted (Fig. 3). However, fluctuations in antibody titers ensued with time in both treated and untreated animals. Following an initial decline between weeks 2 and 10 for each B31-infected animal (Fig. 3A and B), the titers either declined and rose again or remained elevated, as shown for the JD1-infected animals between week 27 and necropsy (Fig. 3C and D). OspC is known to be an immunodominant antigen that is expressed upon transition to the mammalian host environment (19, 40). As such, responses to OspC arose very early (within 2 weeks for most animals) after infection and steadily declined over time among individuals, irrespective of antibiotic treatment (Fig. 4A and B). Importantly, all animals generated an early response to OspC, and antibodies from JD1-infected animals recognized strain B31 OspC.

OppA-2 as a potential diagnostic antigen. The oppA-2 gene belongs to a B. burgdorferi oligopeptide permease operon that is induced during changes in environmental conditions (43). A similar protein has also been used as a vaccine antigen with other bacterial species (41, 46). All infected animals generated anti-OppA-2 antibody responses (Fig. 5). A decline was evident for all three treated strain B31-infected animals shortly after treatment (Fig. 5A and B). This decline was not marked by a return to background levels but, instead, leveled off, and may have even increased, with induction of IgM late in two of three treated animals. An additional 12 animals that were infected with B. burgdorferi strain JD1 all had high-titer anti-OppA-2 antibodies as well (Fig. 5C to F). Here, a decline was seen initially for most
treated animals, whereas the response generally remained elevated in the untreated animals.

**Comparison of EPDTs to different antigens at the peak response and study endpoint.** We also determined the reciprocal endpoint dilution titers of serum antibodies to each antigen from animals at the peak response (16 to 22 weeks) and at necropsy (47 weeks). Figure 6 shows a comparison between the EPDTs of antibodies to the five recombinant antigens and the C6 peptide. The levels of IgG produced in response to DbpA were the highest, and they were lowest for C6. The order of relative responses is DbpA > OspA/OspC/OppA-2 > C6. The percent decline (Table 2) in C6 titer for treated animals was 93.8% for animal GB56 and 98.4% for animals GA59 and FK38. The only other antigens against which antibody titers declined with treatment in all animals but not in both untreated animals were OppA-2 and OspC. For OppA-2, a decline in titers occurred for treated animals GA59 (87.5%) and FK38 (96.9%) but not GB56 (50%). The anti-OspC titers among JD1-infected animals declined for all that were untreated, so the decline among treated animals is not relevant. This will need to be tested on more animals to determine if these changes are statistically significant.

**Early- and late-stage IgM responses.** Longitudinal measurement of the IgM response to each full-length antigen was also included in the assessment (Fig. 7). The anti-OspA IgM responses followed a predictable pattern (Fig. 7A). However, fluctuations in IgM to the other antigens tested, including OspC (Fig. 7B), DbpA (Fig. 7C), and OppA-2 (Fig. 7D) were evident beyond primary infection. For example, treated animal GB56 generated increased anti-OspC IgM levels at weeks 22 and 40. Fluctuations in both the anti-DbpA and anti-OppA-2 IgM response were evident for each of the few animals tested, regardless of antibiotic treatment status.

**DISCUSSION**

The importance of rational design for diagnostic tests is predicated by diversity in the immune responses produced by infected individuals. The use of multiplex testing with cytometric bead-based technology has expanded vigorously in recent years and has become a platform for serological testing. In the case of Lyme disease, the C6 peptide has demonstrated superior reliability as a standalone antigen for diagnostic testing. However, the inclusion of multiple antigens into a serologic test may increase sensitivity. This must be accomplished without compromising test specificity.

To gain insight into antigens that may offer the potential for broad (encompassing all phases of disease) sensitivity, we examined longitudinal responses: (i) in the most appropriate animal model for human Lyme disease, the rhesus macaque, and (ii) both pre- and postantibiotic treatment to include assessment of responses that may be linked to disease resolution. In doing so, we discerned a combination of antigens that would be appropriate for inclusion in a multiantigen serologic test.

In mice, DbpA is known to be a T-cell-independent antigen to which a high proportion of the antibody response is directed (9, 42). It has been tested as a vaccine immunogen (11, 20, 21) and as a diagnostic antigen (35, 45). All NHP infected with either *B. burgdorferi* strain in this study also produced staunch antibody responses to DbpA. It is likely that this is a T-cell-independent an-

![FIG 6 Reciprocal EPDTs for each antigen at the peak response (A) and at the study endpoint (B) for each of the strain B3I-infected macaques. Here, the relative abundance of specific antibodies for each antigen, with and without treatment, can be compared.](image)

**TABLE 2** Percent change in antibody titer with antibiotic treatment

| Treatment status and animal no. | % Change in antibody titer with: |
|---------------------------------|----------------------------------|
|                                 | C6<sup>a</sup> | DbpA  | BBK32 | OspA | OspC | OppA-2 |
| Treated                        |                  |       |       |      |      |        |
| GA59                            | 98.4             | No decline | 75    | 75   | 93.8 | 87.5   |
| FK38                            | 98.4             | 75    | No decline | 87.5 | 87.5 | 96.9   |
| GB56                            | 93.8             | No decline | No decline | 75    | 50   | 50     |
| Untreated                       |                  |       |       |      |      |        |
| GC84                            | No decline       | No decline | 75    | ND<sup>b</sup> | No decline | 50     |
| FT47                            | No decline       | No decline | 50    | 50   | 50   | No decline |

<sup>a</sup> See reference 16.

<sup>b</sup> ND, not done.
tigen for NHP as well. BBK32 is another extracellular matrix
binding protein that has been tested both as a vaccine (11) and as
a diagnostic antigen (35). Though the titer dropped substantially
for one treated B31-infected monkey, only three of the five ma-
caque produced veritable antibody responses to this protein. Due
to the strong and sustained responses to DbpA in all animals tested
to date, it appears a valuable diagnostic antigen. BBK32 has a
lower potential for sensitivity and may not add value to a multi-
plex test.

Despite the artificial induction of primary anti-OspA responses by
inoculation of in vitro cultured spirochetes, we found the fluctuations
in response over time compelling. We detected ospA transcript from
persistently infected macaques (15), so we reasoned that their anti-
OspA antibody responses may not decline predictably over time. In
addition, OspA antibodies have been associated with arthritis during
disseminated infection (3) and have more recently been shown to be
elevated in patients with posttreatment Lyme disease syndrome (12).
For its potential to detect infection at the late disseminated phase,
inclusion of OspA may benefit a multiplex test. OspC is a well-char-
acterized, highly immunogenic B. burgdorferi lipoprotein. Heteroge-
neity in ospC among different isolates and strains has been well doc-
dumented. However, this does not appear to result in type-specific
responses to OspC (23), so it remains a good candidate diagnostic
antigen. Antibody responses to OspC may be expected to arise early
and steadily decline, as this antigen’s expression is known to be shut
off after the advent of the humoral response (28). This is indeed what
we observed.

oppA-2 transcript was detected in heart tissue of mice post-
antibiotic treatment (8). Over a decade ago, two-dimensional
electrophoresis and immunoblotting revealed antibody responses
of infected individuals that targeted an ABC transporter protein of
Borrelia garinii (25). Human patients are known to produce anti-
bodies to the oligopeptide permease A-type protein from B. burg-
dorferi (7, 14, 31). The OppA-2 protein was also demonstrated to
be antigenic in rabbits (13) and in mice (7, 31). The gene is on the
chromosome, whereas the gene from which C6 is derived is lo-
cated on a plasmid that can be lost during infection (39) and
possibly also posttreatment (10). OppA-2 transcript was shown to be
produced by B. burgdorferi in mice postantibiotic treatment, as
an indicator of their metabolic viability. Given the broad reactivity
of macaques to OppA-2 and the decline in response, albeit mod-
erate, posttreatment, this protein could prove to have utility as a
diagnostic antigen both before and after treatment. While we did
not see a response to OppA-2 using preimmune serum, the modest
homology (27) that this protein may have with similar bacterial
proteins necessitates its careful screening with multiple samples
for specificity.

Continuous IgM responses beyond primary introduction of antigen are peculiar but have been observed in B. burgdorferi in-
fec tion previously (1, 22). Primarily through the use of immuno-
blotting, the continual detection of B. burgdorferi antigen-specific
IgM is well documented; however, fluctuations in specific anti-
body levels over time have not generally been presented. The
induction of IgM-producing cells, particularly by T-cell-indepen-
dent antigens (42), may provide a mechanism for such fluctua-
tions and persistent IgM titers.

The results of this study indicate that the antigens OspA, OspC,
DbpA, and OppA-2 may offer distinct benefits when they are
combined with the C6 peptide into a multiantigen diagnostic test.
However, there are limitations to this study, including the inocu-
ulum dose, route, and the small number of animals tested. While
the inoculum dose used here may not accurately reflect the dose
that naturally infected humans would receive, the dynamic re-
sponses to particular antigens may be comparable in a general
sense. Use of an outbred animal model and two different B. burg-
dorferi strains yielded patterns of antibody response that were re-
markably similar per antigen. We cannot assert that these re-
sponses reflect those which may be observed in humans, but
future studies are aimed at testing the responses to these specific
antigens from a broad panel of Lyme disease patients.

FIG 7 Longitudinal IgM responses by macaques infected with B. burgdorferi strain B31 against four different antigens. Shown are the relative responses to OspA (A), OspC (B), DbpA (C), and OppA-2 (D).
For a multiantigen quantitative diagnostic test, specifically the inclusion of OspC could allow for improved sensitivity in the localized phase and that of OspA has the potential to alert a physician about the possibility of development of late arthritis. Detection of OppA-2 increases in antibody levels posttreatment may be a sign of persistent spirochetes or recrudescence as all three treated animals in the strain B31-inoculated experimental group had indications of persistent B. burgdorferi infection (15). The early (~4 weeks p.i.) and strong response to DbpA by a majority of animals indicates that DbpA may also lend diagnostic value to a multiplex test. Observation of dynamic longitudinal responses to various antigens over time may provide insight for optimal diagnostic antigen combination in developing diagnostic tests for other infections with pathogens that possess multiple candidate diagnostic antigens.

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REFERENCES

1. Aguero-Rosenfeld M, et al. 1996. Evolution of the serologic response to Borrelia burgdorferi in treated patients with culture-confirmed erythema migrans. J. Clin. Microbiol. 34:1–9.
2. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. 2005. Diagnosis of Lyme borreliosis. Clin. Microbiol. Rev. 18:484–509.
3. Akin E, McHugh GL, Flavell RA, Kikug E, Steere AC. 1999. The immunoglobulin (IgG) antibody response to OspA and OspB correlates with severe and prolonged Lyme arthritis and the IgG response to P35 correlates with mild and brief arthritis. Infect. Immun. 67:173–181.
4. Asch ES, Bujak DI, Weiss M, Peterson MG, Weinstein A. 1994. Lyme disease: an infectious and postinfectious syndrome. J. Rheumatol. 21:454–461.
5. Bacon RM, et al. 2003. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of Borrelia burgdorferi compared with 2-tiered testing using whole-cell lysates. J. Infect. Dis. 187:1187–1199.
6. Bacon RM, Kugeler KJ, Mead PS. 2008. Surveillance for Lyme disease—United States, 1992–2006. MMWR Surveill. Summ. 57:1–9.
7. Barbour AG, et al. 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.
8. Barthold SW, et al. 2010. Inefficacy of tigecycline against persistent Borrelia burgdorferi. Antimicrob. Agents Chemother. 54:643–651.
9. Barthold SW, Hodzic E, Tunev S, Feng S. 2006. Antibody-mediated disease remission in the mouse model of Lyme borreliosis. Infect. Immun. 74:4817–4825.
10. Bockenstedt L, Mao J, Hodzic E, Barthold S, Fish D. 2002. Detection of attenuated, noninfectious spirochetes in Borrelia burgdorferi-infected mice after antibiotic treatment. J. Infect. Dis. 186:1430–1437.
11. Brown EL, Kim JH, Reisenbichler ES, Hooik M. 2005. Multicomponent Lyme vaccine: three is not a crowd. Vaccine 23:3687–3696.
12. Chandra A, Wormser GP, Marques AR, Latov N, Aaldeani A. 2011. Anti-Borrelia burgdorferi antibody profile in post-Lyme disease syndrome. Clin. Vaccine Immunol. 18:762–771.
13. Crother TR, et al. 2004. Temporal analysis of the antigenic composition of Borrelia burgdorferi during infection in rabbit skin. Infect. Immun. 72:5063–5072.
14. Das S, et al. 1996. Characterization of a 30-kDa Borrelia burgdorferi substrate-binding protein homologue. Res. Microbiol. 147:739–751.
15. Embers ME, et al. 2012. Persistence of Borrelia burgdorferi in rhesus macaques following antibiotic treatment of disseminated infection. PLoS One 7:e29914. doi:10.1371/journal.pone.0029914.
16. Embers ME, Jacobs MB, Johnson BJ, Philipp MT. 2007. Dominant epitopes of the C6 diagnostic peptide of Borrelia burgdorferi are largely inaccessible to antibody on the parent VlsE molecule. Clin. Vaccine Immunol. 14:931–936.
17. Engstrom SM, Shoop E, Johnson RC. 1995. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J. Clin. Microbiol. 33:419–427.
18. Goettner G, et al. 2005. Improvement of Lyme borreliosis serodiagnosis by a newly developed recombinant immunoglobulin G (IgG) and IgM line immunoblot assay and detection of VlsE and DbpA homologues. J. Clin. Microbiol. 43:3602–3609.
19. Grimm D, et al. 2004. Outer-surface protein C of the Lyme disease spirochete: a protein induced in ticks for infection of mammals. Proc. Natl. Acad. Sci. U. S. A. 101:3142–3147.
20. Hagman KE, et al. 2000. Decorin-binding protein A (DbpA) of Borrelia burgdorferi is not protective when immunized mice are challenged via tick infestation and correlates with the lack of DbpA expression by B. burgdorferi in ticks. Infect. Immun. 68:4759–4764.
21. Hanso MS, et al. 1998. Active and passive immunity against Borrelia burgdorferi decorin binding protein A (DbpA) protects against infection. Infect. Immun. 66:2143–2153.
22. Hilton E, Tramontano A, DeVoti J, Sood S. 1997. Temporal study of immunoglobulin M seroreactivity to Borrelia burgdorferi in patients treated for Lyme borreliosis. J. Clin. Microbiol. 35:774–776.
23. Ivanova L, et al. 2009. Comprehensive seroprofiling of sixteen B. burgdorferi OspC: implications for Lyme disease diagnostics. Clin. Immunol. 132:393–400.
24. Jobe DA, et al. 2008. Significantly improved accuracy of diagnosis of early Lyme disease by peptide enzyme-linked immunosorbent assay based on the borreliacidal antibody epitope of Borrelia burgdorferi OspC. Clin. Vaccine Immunol. 15:981–985.
25. Jungblut PR, Grabher G, Stoßfell G. 1999. Comprehensive detection of immunorelevant Borrelia garinii antigens by two-dimensional electrophoresis. Electrophoresis 20:3611–3622.
26. Kalish RA, Leong JM, Steere AC. 1995. Early and late antibody responses to full-length and truncated constructs of outer surface protein A of Borrelia burgdorferi in Lyme disease. Infect. Immun. 63:2228–2235.
27. Kornacki JA, Oliver DB. 1998. Lyme disease-carrying Borrelia species encode multiple lipopolysaccharides homologous to peptide-binding proteins of ABC-type transporters. Infect. Immun. 66:4115–4122.
28. Liang FT, Jacobs MB, Bowers LC, Philipp MT. 2002. An immune evasion mechanism for spirochetal persistence in Lyme borreliosis. J. Exp. Med. 195:415–422.
29. Magnarelli LA, et al. 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.
30. Mogilyansky E, Loo CC, Adelson ME, Mordechai E, Tilton RC. 2004. Comparison of Western immunoblotting and the C6 Lyme antibody test for laboratory detection of Lyme disease. Clin. Diagn. Lab. Immunol. 11:924–929.
31. Nowalk AJ, Gilmore RD, Bowers LC, Philipp MT. 2006. Serologic proteome analysis of Borrelia burgdorferi membrane-associated proteins. Infect. Immun. 74:3864–3873.
32. Nyman D, et al. 2006. VlsE C6 peptide and IgG ELISA antibody analysis for clinical diagnosis of Lyme borreliosis in an endemic area. Clin. Microbiol. Infect. 12:496–497.
33. Pachner AR, et al. 2002. Humoral immune response associated with Lyme borreliosis in nonhuman primates: analysis by immunoblotting and enzyme-linked immunosorbent assay with sonicates or recombinant proteins. Clin. Diagn. Lab. Immunol. 9:1348–1355.
34. Panelius J, et al. 2002. Recombinant OspC from Borrelia burgdorferi sensu stricto, B. afzelii and B. garinii in the serodiagnosis of Lyme borreliosis. J. Med. Microbiol. 51:731–739.
35. Panelius J, et al. 2003. Diagnosis of Lyme neuroborreliosis with antibodies to recombinant proteins DbpA, BBK32, and OspC, and VlsE IR6 peptide. J. Neurol. 250:1318–1327.
36. Philipp MT, Marques AR, Fawcett PT, Dally LG, Martin DS. 2003. C6 test as an indicator of therapy outcome for patients with localized or disseminated Lyme borreliosis. J. Clin. Microbiol. 41:4953–4960.
37. Philipp MT, et al. 2005. A decline in C6 antibody titer occurs in successfully treated patients with culture-confirmed early localized or early disseminated Lyme borreliosis. Clin. Diagn. Lab. Immunol. 12:1069–1074.
38. Piesman J, Hicks TC, Sinsky RJ, Obiri G. 1987. Simultaneous transmission of Borrelia burgdorferi and Babesia microti by individual nymphal Ixodes dammini ticks. J. Clin. Microbiol. 25:2012–2013.
39. Purser JE, Norris SJ. 2000. Correlation between plasmid content and
infectivity in *Borrelia burgdorferi*. Proc. Natl. Acad. Sci. U. S. A. 97:13865–13870.

40. Schwan TG, Piesman J. 2000. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, *Borrelia burgdorferi*, during the chain of infection in ticks and mice. J. Clin. Microbiol. 38:382–388.

41. Tanabe M, et al. 2006. The ABC transporter protein OppA provides protection against experimental *Yersinia pestis* infection. Infect. Immun. 74:3687–3691.

42. Tunev SS, et al. 2011. Lymphadenopathy during Lyme borreliosis is caused by spirochete migration-induced specific B cell activation. PLoS Pathog. 7:e1002066. doi:10.1371/journal.ppat.1002066.

43. Wang X-G, Lin B, Kidder JM, Telford S, Hu LT. 2002. Effects of environmental changes on expression of the oligopeptide permease (opp) genes of *Borrelia burgdorferi*. J. Bacteriol. 184:6198–6206.

44. Wilske B, et al. 1994. Immunoblot using recombinant antigens derived from different genospecies of *Borrelia burgdorferi* sensu lato. Med. Microbiol. Immunol. 183:43–59.

45. Wilske B, Fingerle V, Schulte-Spechtel U. 2007. Microbiological and serological diagnosis of Lyme borreliosis. FEMS Immunol. Med. Microbiol. 49:13–21.

46. Yang M, Johnson A, Murphy TF. 2011. Characterization and evaluation of the *Moraxella catarrhalis* oligopeptide permease A as a mucosal vaccine antigen. Infect. Immun. 79:846–857.