Identification of OppA2 Linear Epitopes as Serodiagnostic Markers for Lyme Disease

Giacomo Signorino,*,‡ Paul M. Arnaboldi,*,‡ Mary M. Petzke,* Raymond J. Dattwyler*,‡,§
Department of Microbiology, New York Medical College, Valhalla, New York, USA; Biopeptides Corp., East Setauket, New York, USA; Department of Medicine, New York Medical College, Valhalla, New York, USA

Lyme disease, the most common vector-borne infectious disease in North America and Europe, is a progressive disease marked by diverse clinical manifestations, which, if untreated, can result in permanent damage to the nervous and musculoskeletal systems (1–5). Prompt treatment with an appropriate antibiotic regimen is highly effective, making accurate early diagnosis essential for preventing bacterial dissemination and late-phase disease manifestations (4, 6–9). Unfortunately, early diagnosis is not always possible. Many of the disease-associated signs and symptoms are nonspecific. The only characteristic sign of Lyme disease is a transient skin lesion, erythema migrans (EM), which appears in the majority of patients during early infection; in areas where Lyme disease is endemic, the presence of an EM lesion is considered virtually diagnostic (1–5, 10). However, EM does not develop in approximately 20% of patients, may go undetected or resolve by the time a patient seeks medical attention, and may be misdiagnosed as a common skin rash, particularly in regions of low endemicity (11–13).

In contrast to most bacterial diseases that can be defined by direct detection of the pathogen, the laboratory diagnosis of Lyme disease is dependent on the demonstration of an antibody response to *Borrelia burgdorferi* (11, 14). In 1995, the Centers for Disease Control and Prevention established the two-tier test approach for the laboratory diagnosis of Lyme disease to address issues of low specificity inherent to early tests (11). This method consists of a sensitive first-tier enzyme-linked immunosorbent assay (ELISA), which, if equivocal or positive, is followed by a second-tier immunoblot assay to provide specificity (11). Most commercially available first-tier ELISAs and all second-tier immunoblots utilize cultured *B. burgdorferi* whole-cell lysates or recombinant proteins as assay targets. The whole-protein antigens in these assays contain a mixture of epitopes, some of which are specific for *B. burgdorferi* while others are conserved cross-reactive epitopes with high structural or linear homology to those found in many other bacterial species (11). As a result, protein-based assays all suffer from an intrinsic lack of specificity. While the two-tier paradigm increases specificity, the corresponding decrease in sensitivity results in these current diagnostic methods being insensitive during the early stages of infection, when treatment is most effective (11–13, 15). A viable approach to circumventing the problem of poor specificity, while maintaining sensitivity, is to develop diagnostic tests based on synthetic antigenic peptides containing specific epitopes (15–21). This strategy allows for the exclusion of nonspecific cross-reactive epitopes while retaining those highly specific for *B. burgdorferi*. An ELISA utilizing an immunodominant peptide of the *B. burgdorferi* VlsE1 protein (C6) has provided proof of this principle by demonstrating increased specificity relative to two-tier tests using whole-cell lysates (16–18).
Studies have demonstrated that relatively few antigens are expressed by *B. burgdorferi* in very early infection. These include FlaB, p66, RevA, oligopeptide permease A1 (OppA1), OppA2, and OppA4 (22–26), with antibodies to OspC (25 kDa), VlsE, BBK32, FlaA (37 kDa), BmpA (39 kDa), FliL, BBG33, LA7, and DbpA proteins appearing slightly later (7, 13, 15, 17, 20, 21, 27, 28). These “early expression” antigens offer attractive targets for the development of improved serodiagnostic methods. In this study, we mapped linear B-cell epitopes of oligopeptide permease A2 (OppA2), the peptide-binding component of the only known peptide transport system in *B. burgdorferi*. OppA2 is expressed early during mammalian infection and elicits antibody responses in mice, rabbits, macaques, and human patients, thereby fulfilling a key requirement for a serodiagnostic marker for early disease (29, 30). The amino acid sequence of OppA2 is highly conserved among all three major pathogenic genospecies of *B. burgdorferi* (31), and antibodies against *B. burgdorferi* OppA2 do not cross-react with Opp proteins from other species, such as *Escherichia coli* (32). Thus, OppA2 presents an attractive target for serodiagnosis. We identified nine immunodominant linear B-cell epitopes of *B. burgdorferi* OppA2 by epitope mapping and used a panel of human sera to assess the serodiagnostic potential of peptide sequences containing each of the identified epitopes. We identified two OppA2 epitopes as sensitive and specific markers for *B. burgdorferi* infection that demonstrate potential as targets in for a seroassay for the laboratory diagnosis of Lyme disease.

**MATERIALS AND METHODS**

**Antibody panels.** All patient samples were collected after obtaining written informed consent from adult volunteers in accordance with protocols approved by the institutional review boards of the respective institutions. A total of 104 sera or plasma samples were obtained from patients presenting with EM at the time of initial visit. Samples were collected from three distinct geographical areas, the Gunderson Lutheran Medical Center in La Crosse, WI (n = 46) (samples generously provided by Steven Callister), the Lyme Disease Diagnostic Center of New York Medical College, Valhalla, Westchester county, NY (n = 34), and the State University of New York at Stony Brook, Long Island, NY (n = 24). Sera from healthy volunteers residing in New Mexico, where Lyme disease is not endemic (n = 45), were purchased from Creative Testing Solutions (Tempe, AZ) and used as negative controls. Sera from patients with a reactive rapid plasma reagin (RPR) test for syphilis (n = 30) or a diagnosis of rheumatoid arthritis (RA) (n = 30) were purchased from Bioreclamation, LLC. All RPR-reactive sera were either equivocal or positive for treponemal antibodies. All negative-control samples that demonstrated positive or equivocal binding to peptides were evaluated with commercially available Lyme disease diagnostic Western blot strip tests to determine if the serum was obtained from an individual with previous Lyme disease. All negative controls included in this evaluation were negative for Lyme disease via Western blot strip tests (Virastripe; Viramed Biotech AG, Planegg, Germany). Samples were stored in aliquots at −80°C.

**Peptides.** Two epitope mapping experiments were performed using overlapping peptide libraries that encompassed the full-length sequence of *B. burgdorferi* B31 OppA2. The first library, consisting of a total of 104 15-mer amino acid (aa) peptides that overlapped by 10 aa (offset by 5 aa), was synthesized by ProImmune, Inc. (Oxford, United Kingdom) using their proprietary ProArray Ultra peptide microarray technology. The library was screened using individual serum samples from eight patients with well-defined *B. burgdorferi* infections, as determined by the presence of 9 to 10 out of 10 bands in a Lyme disease immunostrip assay (Virastripe; Viramed Biotech AG, Planegg, Germany). The second peptide library, consisting of 20-aa peptides overlapping by 15 aa (offset by 5 aa), was generated by Arrayit Corporation (Sunnyvale, CA) using their proprietary peptide microarray technology, and probed using a similar panel of Lyme disease patient sera. Peptides encompassing sequences identified for further analysis were synthesized by LifeTein, Inc. (South Plainfield, NJ) and included OppA (11–25) (amino acids comprising the peptide are shown in parentheses), OppA (191–225), OppA (276–290), OppA (276–300), OppA (286–300), OppA (286–310), OppA (381–400), OppA (356–375), OppA (491–505), OppA (191–225) *Borrelia garinii* variant, OppA (191–225) *Borrelia afzelii* variant, OppA (381–400) variant 1 (found in *B. burgdorferi*, *B. garinii*, and *B. afzelii*), and OppA (381–400) variant 2 (found in *B. garinii* and *B. afzelii*) (Table 1; also see Table S2 in the supplemental material). Amino acid sequences of regions corresponding to OppA (191–225) and OppA (381–400) present in pathogenic *Borrelia* species were aligned using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

**ELISA.** ELISAs were carried out as previously described (19). Peptides were diluted to a concentration of 10 μg/ml in 0.1 M sodium carbonate buffer (pH 9.4), and 50 μl/well was applied to MaxiSorp ELISA plates (Nunc). Wells were blocked overnight at 4°C with 1% bovine serum albumin (BSA) dissolved in phosphate-buffered saline (PBS) (blocking buffer), washed three times with PBS containing 0.05% Tween 20 (PBS-T), and incubated for 2 h at room temperature with human plasma or serum samples diluted 1:100 in blocking buffer. Each plate included samples from patients and healthy donors, and all samples were assayed in triplicate. After washing, wells were incubated for 1 h at room temperature (RT) with a 1:15,000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-human IgG and IgM (Jackson ImmunoResearch). Reactions were developed with 3-tetramethylbenzidine membrane (TMB) substrate (KPL, Gaithersburg, MD) for 30 min and stopped by the addition of 2 N sulfuric acid. Optical densities were determined at 450- and 570-nm wavelengths using a SpectraMax Plus plate reader (Molecular Devices). Absorbance was determined by subtracting the value obtained at 570 nm from that obtained at 450 nm. The cutoff for unequivocal positivity was defined as three standard deviations (SD) above the mean absorbance of the healthy control samples. A sample with a mean absorbance that was between 2 SD and 3 SD above the mean of the healthy donor samples was defined as equivocal, and a sample with mean absorbance <2 SD above the mean of the healthy donor samples was considered negative.

**Statistics.** Statistical analysis was performed using GraphPad Prism 6.0 software (San Diego, CA). The sensitivity and specificity of each peptide were determined by comparing absorbance values from Lyme disease patients with values from negative-control groups by receiver operating characteristic (ROC) analysis using 3 SD from the mean of healthy controls as the cutoff. Differences in the mean absorbance values obtained using Lyme disease and negative-control groups were compared by one-way analysis of variance (ANOVA) followed by a Tukey’s post hoc test for multiple comparisons. A chi-square test was performed to directly compare...
pare unequivocally positive (3 SD above the mean absorbance of healthy donor samples) and negative (<2 SD above the mean absorbance of healthy donor samples) results for each peptide. Differences were at considered statistically significant at P values of <0.05.

RESULTS
Identification of immunogenic epitopes of OppA2. In order to define the immunodominant epitopes of *B. burgdorferi* OppA2, we performed two separate epitope mapping studies using high-titer serum samples from eight Lyme disease patients (19). Two peptide libraries were synthesized and screened in order to provide independent validation of the results. The results of each epitope mapping study were reviewed independently. Multiple epitopes of OppA2, a large protein of 528 aa, were detected by each serum sample in both studies. Further analysis was confined to those epitopes that were detected by a minimum of six of the eight Lyme disease patient samples (75%) in each mapping study. The results of the two studies were largely consistent, although there were some minor disparities that likely resulted from differences in peptide lengths and overlaps as well as sensitivities in detection systems. In both studies, analysis demonstrated that the epitopes were not uniform in length and often spanned contiguous peptides, though it was not possible to determine whether these areas contained a single epitope, overlapping epitopes, or adjacent epitopes. The region of OppA2 consisting of amino acids 276 to 310 had the highest rate of recognition; antibody binding was observed with 7 of 8 Lyme disease patient sera in the first epitope mapping study, and with 8 of 8 Lyme disease patient sera in the second study. However, not all samples bound to the same peptide sequence, which suggested the presence of more than one epitope in this region. Based on this observation, multiple peptides encompassing this region were synthesized. The following nine peptides, encompassing epitopes from five different regions spanning the length of OppA2, were selected for synthesis and further assessment as potential serodiagnostic targets: OppA (11-25), OppA (191-225), OppA (276-290), OppA (276-300), OppA (286-300), OppA (286-310), OppA (381-400), OppA (356-375), and OppA (491-505) (Table 1 and Fig. 1).

OppA2 peptides react specifically with antibodies from Lyme disease patients. In order to confirm the immunoreactivity of the identified OppA2 epitopes, the nine peptides were screened by ELISA using serum or plasma obtained from 104 patients with physician-diagnosed Lyme disease based on the presence of one or more EM lesion(s). Samples were obtained from three distinct geographical regions where Lyme disease is endemic. Control sera were obtained from healthy donors residing in New Mexico, a region of the United States where Lyme disease is not endemic. Each peptide was assessed in triplicate wells, and results were validated in two independent ELISAs. A sample was defined as unequivocally positive if the mean absorbance of the three replicate wells was greater than three standard deviations (SD) above the mean absorbance of the healthy donor samples assessed on the same plate. A reaction was considered to be equivocal if the mean absorbance was between 2 SD and 3 SD above the mean of the healthy donors and negative if the mean absorbance was <2 SD above the mean of the healthy donor samples. Peptides OppA (11-25) and OppA (491-505) reacted comparably with antibodies from Lyme disease patients and from healthy donors (data not shown), indicating the presence of cross-reactive epitopes that are not unique to *Borrelia*; these peptides were excluded from further analyses. The remaining seven peptides were detected by no more than 4.5% of the healthy donor samples but gave unequivocally positive reactions with between 15.4% and 45.2% of Lyme disease patients.
TABLE 2 Comparative ELISA assessment of OppA2 peptides as serodiagnostic markers for Lyme disease

| Peptide (amino acid sequence) | Reaction result | Antibody panel results (% [n]) of samples from: |
|------------------------------|----------------|-----------------------------------------------|
|                              |                | All Lyme disease patients (n = 104) | Healthy donors (n = 44) | RA* patients (n = 30) | Syphilis patients (n = 30) |
| OppA (191-225)               | Positive       | 44.2 (46) | 4.2 (2) | 0 (0) | 11.5 (3) |
|                              | Equivocal       | 17.3 (18) | 0 (0) | 0 (0) | 7.2 (2) |
|                              | Negative       | 38.5 (40) | 95.5 (42) | 100 (30) | 80.8 (21) |
| OppA (381-400)               | Positive       | 40.4 (42) | 4.2 (2) | 0 (0) | 7.2 (2) |
|                              | Equivocal       | 16.3 (17) | 2.3 (1) | 0 (0) | 3.8 (1) |
|                              | Negative       | 43.3 (45) | 93.2 (41) | 100 (30) | 88.5 (23) |

* At least 3 SD from the mean absorbance of healthy donor samples.
* Between 2 SD and 3 SD from the mean absorbance of healthy donor samples.
* Less than 2 SD from the mean absorbance of healthy donor samples.
* All Lyme disease patients presented with one or more erythema migrans lesion(s) and resided in geographical regions where Lyme disease is endemic.
* RA, rheumatoid arthritis.

The sensitivity and specificity of peptides OppA (191-225) and OppA (381-400) for identifying anti-\textit{Borrelia} antibodies in patient samples were determined by ROC analysis using a cutoff of 3 SD from the mean absorbance of the healthy control samples. When comparing detection of Lyme disease patient samples to that of only healthy controls, OppA (191-225) demonstrated 95.45% specificity and 45.19% sensitivity, and OppA (381-400) displayed 95.45% specificity and 39.42% sensitivity. The area under the curve (AUC) for both samples was comparable (0.8654 versus 0.8639, respectively). When ROC analysis was performed comparing samples from Lyme disease patients with samples from both healthy and disease controls (healthy, syphilis, and RA), specificity and sensitivity values were 45.19% and 96% (AUC, 0.8089) for OppA (191-225) and 39.42% and 96% (AUC, 0.8129) for OppA (381-400). Therefore, both OppA2 peptides displayed highly specific recognition of anti-\textit{Borrelia} antibodies with respect to healthy and disease controls.

\begin{enumerate}
\item \textbf{OppA (191-225) and OppA (381-400) linear epitopes are conserved among different pathogenic \textit{Borrelia} species.} Lyme disease in North America is exclusively caused by \textit{B. burgdorferi} sensu stricto, while in Europe, there are multiple pathogenic genspecies; \textit{B. garinii} and \textit{B. afzelii} are the most common, but infection with \textit{B. burgdorferi} sensu stricto, \textit{Borrelia valaisiana}, and \textit{Borrelia lusitaniae} are not uncommon. To determine whether the sequences of OppA (191-225) and OppA (381-400) are conserved among pathogenic \textit{Borrelia}, a BLAST sequence comparison was performed. Amino acid sequence alignment of regions corresponding to OppA (191-225) and OppA (381-400) encoded by three \textit{B. burgdorferi}, three \textit{B. garinii}, and two \textit{B. afzelii} strains revealed a high degree of sequence conservation (Fig. 3). Although differences in single amino acids were present among the different \textit{Borrelia} species, 70% sequence homology was observed for OppA (191-225), with 19 identical and 7 similar amino acids (out of 35 amino acids). OppA (381-400) shared 80% sequence homology among genspecies, with 75% (15 of 20 amino acids) identity and 5% (1 of 20 amino acids) similarity. To evaluate the extent of amino acid variability on antibody recognition in Lyme disease patients, we selected two peptide variants for each OppA (191-225) and OppA (381-400) (see Table S2 in the supplemental material). The OppA (191-225) linear epitope demonstrated a high degree of sequence conservation (Fig. 3). For OppA (191-225), one variant was selected from a strain of \textit{B. garinii} [OppA (191-225) \textit{B. garinii} variant] and the other from \textit{B. afzelii} [OppA (191-225) \textit{B. afzelii} variant], while only two variants were identified for OppA (381-400), variant 1 (found in three pathogenic species) and variant 2 (found in \textit{B. garinii} and \textit{B. afzelii} only) (see Table S2 in the supplemental material). The OppA (191-225) \textit{B. afzelii} variant demonstrated reduced positive detection of Lyme disease sera compared to the parent OppA (191-225) peptide sequence, while all other variant peptides produced similar detection profiles compared to their respective parent peptides (see Fig. S1 and Table S3 in the supplemental material). The data suggest that the tyrosine in position 1 and/or the valine in position 30 are critical for antibody binding as these are the only two amino acids that differ from both OppA (191-225) and OppA (191-225) \textit{B. garinii} variant, yet positive recognition was greatly reduced compared to both (see Table S3 in the supplemental material). These results demonstrate that the OppA (191-225) and OppA (381-400) immunodominant epitopes of OppA2 are highly conserved among disease-causing \textit{Borrelia} species; however, due to these minor variations, it may be necessary to use an
\end{enumerate}
alternate sequence for OppA (191-225) where European strains of Borrelia dominate.

**DISCUSSION**

Specificity is a major problem for all whole-protein-based seroassays. Antigens expressed by *B. burgdorferi* contain epitopes that are unique to *B. burgdorferi*, as well as epitopes similar to those expressed by other bacteria. As a consequence, assays using whole-protein antigens (Ags), either natural or recombinant, are inherently constrained; as sensitivity is increased, specificity is unacceptably reduced. This affects serodiagnosis in both early and late Lyme disease, but in early disease, when antibody (Ab) responses are just beginning, these constraints are crucial. This helps explain why present seroassays are widely deemed to be inadequate.
Synthetic peptide-based diagnostic tests, containing epitopes that are unique to *Borrelia*, present a promising alternative to whole-protein assays. The IR6 assay, based on a single epitope contained in a conserved region of VlsE, has demonstrated the feasibility of peptide-based assays and has been suggested as an alternative to the current two-tier assay (18). However, C6 does not bind IgM particularly well, VlsE is not expressed until infection is fully established, and the sequence has more variability than originally thought (16,17, 20, 33, 34). Thus, it is unlikely to supplant the current CDC two-tier paradigm. Additional linear diagnostic epitopes have been identified in OspC and BBK07 (19, 21, 35, 35), and it has been demonstrated that combining an OspC peptide (PepC10) with the IR6 epitope improves sensitivity (36).

Although single- or dual-peptide-based serological assays offer promise, it is unlikely that one or two epitope targets can overcome the intrinsic variability in the human immune response and bacterial antigens; small variations in the amino acid sequence of the single diagnostic epitope could reduce the ability of the peptide to be recognized by patient sera. An analysis by Barbour et al. demonstrated that a serodiagnostic test with appropriate sensitivity and specificity would require a minimum of five antigens (22). One or more peptides from OppA2 could play an important part in such a multipeptide assay. *B. burgdorferi* OppA2, a surface-localized lipoprotein, possesses several key characteristics required of an effective serodiagnostic marker. Multiple studies have established that OppA2 is expressed, and elicits an antibody response, early during the course of mammalian infection. Transcriptional induction of *oppA2* has been observed in mouse tissues by day 14 postinfection (29, 37), and OppA2 protein was detected from day 7 through day 14 of infection in a rabbit skin infection model (30). Embers and colleagues demonstrated that *B. burgdorferi* OppA2 is an immunodominant antigen in nonhuman primates and proposed that an assay based on a combination of OppA2, DbpA, OspC, OspA, and the C6 peptide would have the potential for detecting Lyme disease at all stages (38). In the present study, we used blood samples from patients with well-defined early Lyme disease to perform epitope mapping of OppA2. We identified immunodominant linear epitopes in five regions of the protein, and screened nine peptides that were generated using sequences from these regions. Two peptides, OppA (191-225) and OppA (381-400), demonstrated potential as diagnostic markers for Lyme disease.

Defining and analyzing linear epitopes, as we did with OppA2, provides a means to effectively address two of the major issues in selecting targets for the serodiagnostics of Lyme disease: cross-reactivity with similar epitopes expressed by other bacteria and sequence variability of the target epitope in various *B. burgdorferi* isolates. In this study, potentially cross-reactive epitopes were identified and eliminated by performing a BLAST search to assess the degree of homology of defined linear epitopes with sequences
from other organisms. Both OppA (191-225) and OppA (381-400) demonstrated specificity for anti- Borrelia antibodies with respect to healthy controls, patients with a related spirochetal infection (syphilis), or patients with rheumatoid arthritis, an inflammatory disorder with similar manifestations to Lyme arthritis. The syphilis patient samples found to be positive or equivocal for OppA (381-400) were also positive or equivocal for OppA (191-225) and included the single positive sample for OppA (276-290). In addition, these three serum samples generated the highest absorbance values among syphilis patient sera for the remaining OppA peptides and were often just below the threshold for equivocal detection. Different healthy control sera were to be found positive or equivocal for OppA (191-225) and OppA (381-400). Reactivity among the few negative-control sera could indicate the presence of a contaminating factor or a proclivity for nonspecific adsorption of antibody to the peptide-coated plates that is specific to these serum samples. The lack of binding in the majority of other control sera suggests that nonspecific interactions are not attributable to the peptides. As binding was not observed with the majority of other syphilis sera, a specific response to a T. pallidum antigen is also unlikely. Alternatively, as some of the negative disease controls (syphilis and RA) were obtained in areas where Lyme disease is endemic, it is also possible that these individuals had prior Lyme disease or were exposed to Borrelia.

The BLAST search also enabled assessment of the degree of sequence variability among B. burgdorferi genotypes. Lyme disease is caused by infection with B. burgdorferi in North America and most commonly by B. burgdorferi, B. afzelii, and B. garinii in Europe and Asia. A diagnostic test intended for use beyond North America would require the incorporation of epitopes that would detect infections by all three pathogenic Borrelia genospecies. The nucleotide sequence of oppA2 is highly conserved among Lyme disease-causing Borrelia, but not in species that cause relapsing fever (B. turicatae, B. hermsii, and B. anserina) (31). B. burgdorferi B31 peptides OppA (191–225) and OppA (381–400) were found to share 74 to 80% amino acid sequence homology with multiple strains of B. burgdorferi, B. afzelii, and B. garinii (Fig. 3). However, we found that variations in the amino acid sequence of OppA (191–225) in a strain of B. afzelii greatly reduced positive binding to the peptide (see Fig. S1 in the supplemental material). Therefore, in regions where European strains of Borrelia predominate, it may be necessary to include an alternate sequence for OppA (191–225). In contrast to several other potential serodiagnostic antigens, which are encoded on plasmids, the gene for OppA2 is located on the chromosome (31, 39). This characteristic precludes concerns due to potential plasmid loss or instability that could result in loss of expression.

In conclusion, we have identified two-peptide epitopes derived from the OppA2 outer membrane protein of B. burgdorferi that demonstrate potential as targets in a seroassay for the laboratory diagnosis of Lyme disease. Further identification of unique epitopes of B. burgdorferi proteins is necessary to facilitate the development of a highly specific and sensitive serological assay for Lyme disease diagnosis.

ACKNOWLEDGMENTS

We thank Steven Callister for generously providing serum samples for this study.

The peptides presented in this article are protected under U.S. provisional patent application no. 61/705,344, filed by Biopeptides Corp., and could possibly serve as a future source of funding for Biopeptides Corp. R.J.D. is a shareholder in Biopeptides Corp. P.M.A. has a research appointment with Biopeptides Corp. S.C., G.S., and M.M.P. have no conflicts to declare.

This work was supported by National Institute of Allergy and Infectious Diseases grants R44 AI074092 and R43 AI102435.

REFERENCES

1. Steere AC. 2001. Lyme disease. N. Engl. J. Med. 345:115–125. http://dx.doi.org/10.1056/NEJM200107123450207.
2. Stanek G, Wormser GP, Gray J, Strle F. 2012. Lyme borreliosis. Lancet 379:461–473. http://dx.doi.org/10.1016/S0140-6736(11)60037-7.
3. Arch ES, Bujak DI, Weiss M, Peterson MD, Weinstein A. 1994. Lyme disease: an infectious and postinfectious syndrome. J. Rheumatol. 21: 454–461.
4. Kowalski TJ, Tata S, Berth W, Mathiason MA, Agger WA. 2010. Antibiotic treatment duration and long-term outcomes of patients with early Lyme disease from a Lyme disease-hyperendemic area. Clin. Infect. Dis. 50:512–520. http://dx.doi.org/10.1086/649920.
5. Dattwyler RJ. 2010. A commentary on the treatment of early Lyme disease. Clin. Infect. Dis. 50:521–522. http://dx.doi.org/10.1086/649921.
6. Coyle PK, Schutzer SE. 2002. Neurologic aspects of Lyme disease. Med. Clin. North Am. 86:261–284. http://dx.doi.org/10.1016/S0000-0011-156-5.
7. Halperin JJ. 2009. Nervous system Lyme disease: diagnosis and treatment. Curr. Neurol. Dis. 6:4–12. http://dx.doi.org/10.1016/j.cnd.2009.03.020-x.
8. Nardelli DT, Callister SM, Schell RF. 2008. Lyme arthritis: current concepts and a change in paradigm. Clin. Vaccine Immunol. 15:21–34. http://dx.doi.org/10.1128/CVI.00330-07.
9. Steere AC, Schoen RT, Taylor E. 1987. The clinical evolution of Lyme arthritis. Ann. Intern. Med. 107:725–731.
10. Nadelman RB, Wormser GP. 1998. Lyme borreliosis. Lancet 352:557–565. http://dx.doi.org/10.1016/S0140-6736(98)01146-5.
11. Centers for Disease Control and Prevention (CDC). 1995. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Dis. MMWR Mortal. Wkly. Rep. Re:4590–391.
12. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. 2005. Diagnosis of Lyme borreliosis. Clin. Microbiol. Rev. 18:484–509. http://dx.doi.org/10.1128/CMR.18.3.484-509.2005.
13. Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Kligerman MS, Krause PJ, Bakken JS, Strle F, Stange B, Bockenstedt L, Fish D, Dumler JS, Nadelman RB. 2006. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin. Infect. Dis. 43:1089–1134. http://dx.doi.org/10.1086/508667.
14. Centers for Disease Control and Prevention (CDC). 1997. Lyme disease—United States, 1996. Morb. Mortal. Wkly. Rep. 46:531–535.
15. Wormser GP, Aguero-Rosenfeld ME, Nadelman RB. 1999. Lyme disease serology: problems and opportunities. JAMA 282:79–80.
16. Liang FT, Steere AC, Marques AR, Johnson BJ, Miller JN, Phillip MT. 1999. Sensitive and specific serodiagnosis of Lyme disease by 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. http://dx.doi.org/10.1086/374395.
17. Bacon RM, Biggerstaff BJ, Schriefer ME, Gilmore RD, Jr, Philip MT, Steere AC, Wormser GP, Marques AR, Johnson BJ. 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. http://dx.doi.org/10.1086/374395.
18. Wormser GP, Schriefer M, Aguero-Rosenfeld ME, Levin A, Steere AC, Nadelman RB, Nowakowski J, Marques A, Johnson BJ, Dumler JS. Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease. Diagn. Microbiol. Infect. Dis. 75:9–15. http://dx.doi.org/10.1016/j.diagmicrobio.2012.09.003.
19. Arnaboldi PM, Smedsaa R, Sambir M, Callister SM, Imperato JA, Dattwyler RJ. 2013. Outer surface protein C peptide derived from Borrelia burgdorferi sensu stricto as a target for serodiagnosis of early Lyme disease. Clin. Vaccine Immunol. 20:474–481. http://dx.doi.org/10.1128/CVI.00608-12; http://dx.doi.org/10.1128/CVI.00608-12.
20. Gomes-Solecki MJ, Meirelles L, Glass J, Dattwyler RJ. 2007. Epitope length, genospecies dependency, and serum panel effect in the IR6 enzyme-linked immunosorbent assay for detection of antibodies to Borrelia burgdorferi. Clin. Vaccine Immunol. 14:875–879. http://dx.doi.org/10.1128/CVI.00122-07.

21. Coleman AS, Rossmann E, Yang X, Song H, Lamichhane CM, Iyer R, Schwartz J, Pal U. 2011. BBK07 immunodominant peptides as serodiagnostic markers of Lyme disease. Clin. Vaccine Immunol. 18:406–413. http://dx.doi.org/10.1128/CVI.00461-10.

22. 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.

23. Engstrom SM, Shoop E, Johnson RC. 1995. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J. Clin. Microbiol. 33:419–427.

24. Magnarelli LA, Ijdo JW, Padula SJ, Flavell RA, Fikrig E. 2000. Serologic diagnosis of Lyme borreliosis by using enzyme-linked immunosorbent assays with recombinant antigens. J. Clin. Microbiol. 38:1735–1739.

25. Nowalk AJ, Gilmore RD, Jr, Carroll JA. 2008. Effects of environmental changes on expression of the oligopeptide permease (opp) genes of Borrelia burgdorferi. J. Bacteriol. 184:6198–6206. http://dx.doi.org/10.1128/JB.184.22.6198-6206.2002.

26. Crother TR, Champion CI, Whitelegge JP, Aguilera R, Wu XY, Blanco DR, Miller JN, Lovett MA. 2004. Temporal analysis of the antigenic composition of Borrelia burgdorferi during infection in rabbit skin. Infect. Immun. 72:5063–5072. http://dx.doi.org/10.1128/IAI.72.9.5063-5072.2004.

27. Kornacki JA, Oliver DB. 1998. Lyme disease-causing Borrelia species encode multiple lipoproteins homologous to peptide-binding proteins of ABC-type transporters. Infect. Immun. 66:4115–4122.

28. Lin B, Short SA, Eskildsen M, Klemptner MS, Hu LT. 2001. Functional testing of putative oligopeptide permease (Opp) proteins of Borrelia burgdorferi: a complementation model in opp(−) Escherichia coli. Biochim. Biophys. Acta 1499:222–231. http://dx.doi.org/10.1016/S0167-4889(00)00121-X.

29. Ohnishi J, Schneider B, Messer WB, Piesman J, de Silva AM. 2003. Genetic variation at the vlsE locus of Borrelia burgdorferi within ticks and mice over the course of a single transmission cycle. J. Bacteriol. 185:4432–4441. http://dx.doi.org/10.1128/JB.185.15.4432-4441.2003.

30. Bykowski T, Woodman ME, Cooley AE, Brissette CA, Wallich R, Brade V, Kraiczcy P, Stevenson B. 2008. Borrelia burgdorferi complement regulator-acquiring surface proteins (BbCRASPs): expression patterns during the mammal-tick infection cycle. Int. J. Med. Microbiol. 298(Suppl 1):249–256. http://dx.doi.org/10.1016/j.ijmm.2007.10.002.

31. Mathiesen MJ, Christiansen M, Hansen K, Holm A, Åsbrink E, Theisen M. 1998. Peptide-based OspC enzyme-linked immunosorbent assay for serodiagnosis of Lyme borreliosis. J. Clin. Microbiol. 36:3474–3479.

32. Burbelo PD, Issa AT, Ching KH, Cohen JI, Iadarola MJ, Marques A. 2010. Rapid, simple, quantitative, and highly sensitive antibody detection for Lyme disease. Clin. Vaccine Immunol. 17:904–909. http://dx.doi.org/10.1128/CVI.00476-09; http://dx.doi.org/10.1128/CVI.00476-09.

33. Hodzic E, Feng S, Freet JK, Barthold SW. 2003. Borrelia burgdorferi population dynamics and prototype gene expression during infection of immunocompetent and immunodeficient mice. Infect. Immun. 71:5042–5055. http://dx.doi.org/10.1128/IAI.71.9.5042-5055.2003.

34. Embers ME, Hasenkampf NR, Jacobs MB, Philipp MT. 2012. Dynamic longitudinal antibody responses during Borrelia burgdorferi infection and antibiotic treatment of rhesus macaques. Clin. Vaccine Immunol. 19:1218–1226. http://dx.doi.org/10.1128/CVI.00228-12; http://dx.doi.org/10.1128/CVI.00228-12.

35. Bonolo JI, Tilly K, Stevenson B, Hogan D, Rosa P. 1998. Oligopeptide permease in Borrelia burgdorferi: putative peptide-binding components encoded by both chromosomal and plasmid loci. Microbiology 144(Pt 4):1033–1044.