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Establishment of Enzyme-Linked Immunosorbent Assay Using Purified Recombinant 83-Kilodalton Antigen of *Borrelia burgdorferi* Sensu Stricto and *Borrelia afzelii* for Serodiagnosis of Lyme Disease

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The 83-kDa antigen of *Borrelia burgdorferi* was expressed as a recombinant protein in *Escherichia coli* and purified for use in an enzyme-linked immunosorbent assay (p83-ELISA). Antibodies to the 83-kDa antigen of both the immunoglobulin G (IgG) and IgM isotypes could be detected in all stages of Lyme disease. Sensitivity varied, depending on the clinical stage of illness. In early stages, as defined for 118 patients with erythema migrans, it was found to be 20% (24 of 118 patients: 7 with IgM, 16 with IgG, and 1 with IgM and IgG). Of the patients with late-stage Lyme arthritis and acrodermatitis chronica atrophicans, 94% (16 of 17: 2 with IgM and IgG and 14 with IgG) and 86% (36 of 42: 2 with IgG and IgM and 34 with IgG) revealed positive results in the p83-ELISA, respectively. p83 displays sequence heterogeneity according to the genomospecies, but when the reactions of serum specimens from acrodermatitis chronica atrophicans patients and arthritis patients with p83 derived from representative strains of *B. burgdorferi* sensu stricto and *Borrelia afzelii* in ELISAs were compared, no differences in specificity and sensitivity were seen. When 82 serum specimens from healthy controls were tested, none had IgG and only 3 (4%) had IgM antibodies, indicating a high specificity. Positive reactions with antibodies against *Treponema pallidum* (1 of 37 patients; IgG) and Epstein-Barr virus (1 of 44 patients; IgM) and with autoantibodies of various specificities (1 of 53 patients; IgG) were seen with <3% of the serum samples tested, confirming the high specificity for *B. burgdorferi*. In conclusion, with a sensitivity of <13% for IgM antibodies, the IgM p83-ELISA provided little diagnostic information for Lyme disease, whereas the IgG p83-ELISA appears to be a suitable test for serodiagnosis of advanced-stage Lyme disease.

The most common methods for laboratory diagnosis of Lyme disease are serological tests (12, 29). Assays which utilize whole cells or extracts thereof as antigens have adequate sensitivity but are not satisfactory with respect to specificity (2, 10). To increase specificity, efforts have been undertaken to establish assays with isolated, single antigens, either native or recombinant (11, 13–15, 23, 26, 31, 33). In later stages of Lyme disease, antibodies are frequently reactive with a high-molecular-mass protein (33, 35, 36) which was reported to be a strong immunogen and, in culture, a poorly expressed antigen of *Borrelia burgdorferi* called p83, p93, p94, or p100 (17–21, 25, 30). There is evidence, based on sequence comparisons, that p83, p94, and p100 are homologs (6). The cellular function and possible pathogenic implications of this protein have not been finally clarified (9, 20, 21, 25).

There is a broad consensus in the literature, based on results on Western blotting (immunoblotting), that p83 and its homologs are highly specific (4, 8, 22, 31, 33) and useful markers (35, 36) for late-stage Lyme disease. In advanced Lyme borreliosis, clinical symptoms may be variable (2). Highly specific serologic assays are therefore desirable. Some authors claim that Western blots employing either native (8, 22, 34, 35) or recombinant (31, 32) antigens are sufficiently sensitive and specific. The Western blot, however, is a nonquantitative method and results are observer dependent, especially in cases of faint bands. There is some controversy regarding interpretation criteria for Western blots (8, 16, 22, 36). The enzyme-linked immunosorbent assay (ELISA), in contrast, is quantitative. In this study, we established an ELISA using purified recombinant p83 as an antigen (p83-ELISA) and evaluated its suitability for routine diagnostic use.

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MATERIALS AND METHODS

Bacterial strains. *B. burgdorferi* sensu stricto strain GeHo and *Borrelia afzelii* Bo23 (passage numbers, >25) (both provided by K. Pelz, Institut für Medizinische Mikrobiologie und Hygiene, Freiburg, Germany) were grown in modified Kelly's medium (3) at 33°C for 4 days. Spirochetes were centrifuged at 5,000 × *g* for 30 min at 20°C and used for isolation of DNA as previously described (26).

Escherichia coli Topp2 (Stratagene) was used for transformation and expression of the recombinant protein.

Construction of recombinant p83. Recombinant p83 was constructed as previously described (26). Oligonucleotides (Fig. 1) for amplification of the entire open reading frame by PCR were derived from the published sequence of *B. burgdorferi* B31 (25) and the sequence of *B. afzelii* PKo (17). The product of PCR was ligated into expression vector pJLA602 (medac).

Expression and purification of recombinant p83 from *B. burgdorferi* GeHo. *E. coli* Topp2 expressing recombinant p83 was grown overnight in Luria broth medium (GIBCO) containing 100 μg of ampicillin per ml. The culture was then diluted 1:50 in the same medium and grown for 3 additional h at 37°C. Expression of p83 was induced by raising the temperature to 42°C for 7 h. The cells were then centrifuged at 5,000 × *g* for 15 min and washed twice in phosphate-buffered saline. After the final wash, the cells were resuspended in buffer containing 8 M urea, 20 mM NaCl, 20 mM Tris HCl (pH 7.0), 5 mM benzamidine, 5 mM tetrathionate, 5 mM EDTA, and 1 μM leupeptin, and the mixture was stirred for 2 h at 4°C and centrifuged for 30 min at 27,000 × *g*. The supernatant was dialyzed against start buffer A, containing 1 M ammonium sulfate, 0.5 M Na_2SO_4, 20 mM Tris HCl (pH 6.0), 5 mM benzamidine, 5 mM tetrathionate, 5 mM EDTA, and 1 μM leupeptin. After a second centrifugation step for 30 min at 27,000 × *g*,

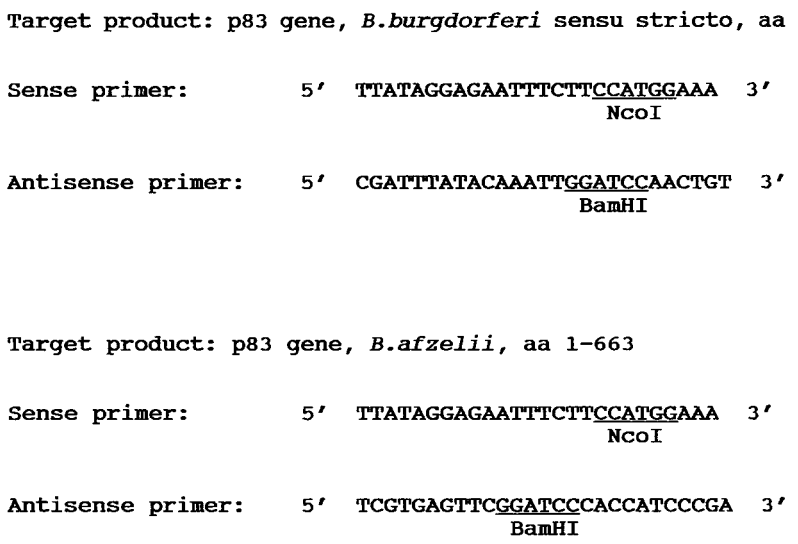

FIG. 1. Sets of primers for amplification of the p83 genes from *B. burgdorferi* sensu stricto strain GeHo and *B. afzelii* Bo23. Cutting sites of restriction endonucleases *Nco*I and *Bam*HI are underlined. aa, amino acids.

hydrophobic-interaction chromatography was performed with phenyl-Sepharose HP HiLoad 16/10 (Pharmacia). The elution buffer was the same as start buffer A but without ammonium sulfate. Fractions containing p83 were dialyzed against 8 M urea–20 mM NaCl–20 mM Tris HCl (pH 7.0)–5 mM benzamidine–5 mM tetrathionate–5 mM EDTA–1 μM leupeptin prior to performance of gel filtration with Superdex 200 (Pharmacia). The protein content was determined by using Coomassie Plus protein assay reagent (Pierce).

ELISA. The ELISA was done by standard methods. Flat-bottomed 96-well microtiter plates (Greiner) were coated at 4°C overnight with 100 μl of purified recombinant p83, diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$ [pH 9.5]) to 0.5 μg/ml, per well. After being washed with 0.05% (vol/vol) Tween 20 in washing buffer (0.15 M NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 1 mM KH_2PO_4), the plates were incubated with patient sera, diluted 1:800 for immunoglobulin G (IgG) and 1:100 for IgM in washing buffer containing 1% (wt/vol) Pentex (Bayer) and 2% (vol/vol) Tween 20, for 1 h at 37°C. The plates were then washed again. Bound antibodies were detected by peroxidase-conjugated goat anti-human IgM and IgG (diluted 1:30,000 in the same buffer as the sera and incubated for 30 min at 37°C). After a third washing step, substrate solution (0.4 mg of phenylenediamine per ml, in tablets; Dako) in substrate buffer (20 mM Na_2HPO_4, 100 mM KH_2PO_4) was added and the mixture was incubated for 15 min in a dark chamber at 4°C. The reaction was stopped with 2 M H_2SO_4, and the optical density (OD) at 492 nm was read with an SLT reader (type 340 ATC). The cutoff for OD readings at 492 nm was set at 2 standard deviations above the mean of 154 healthy control samples.

p83 from *B. afzelii* Bo23. Expression and purification of p83 and the ELISA were done as with p83 from strain GeHo (see above).

Patient serum samples. A total of 210 serum samples from patients with Lyme disease were obtained from the Department of Neurology and Internal Medicine of the University Hospital of Freiburg and from the Department of Dermatology of the University Hospital of Munich. All patients were clinically diagnosed as having typical manifestations of Lyme disease; they included 118 patients with erythema migrans (EM), 33 patients with different stages of neuroborreliosis (NB), 17 patients with Lyme arthritis (ART), and 42 patients with acrodermatitis chronica atrophicans (ACA). Thirty NB patients were in stage II and three were in stage III of Lyme disease, according to the following criteria (18): stage II, symptoms persisting less than 6 months (acute NB); stage III, symptoms persisting more than 6 months (chronic NB). The diagnoses of all patients were confirmed by positive reactions in routine serology, defined as follows: (i) a titer of >320 in a passive hemagglutination assay using a mixture of antigens from three strains representing the genomospecies of *B. burgdorferi* sensu lato and (ii) a positive titer (IgM > 48, IgG > 128) in an immunofluorescent assay employing whole cells of *B. burgdorferi* GeHo as an antigen. In routine serology, serum samples were preabsorbed with an ultrasonicate supernatant of *Treponema phagedenis* cells. This should have reduced cross-reactivity of antibodies.

To estimate the cutoff value of the p83-ELISA, 154 serum samples from local students with no current clinical symptoms and with no prior history of Lyme disease were also tested. To confirm specificity, 82 additional serum specimens which fulfilled the same criteria were tested. To investigate cross-reactivity of p83 with antibodies induced by polyclonal stimulation due to Epstein-Barr virus (EBV) infection, 44 serum samples from patients with acute EBV infection were tested. Furthermore, 37 serum samples from patients with syphilis and 53 serum samples from patients with autoantibodies of various specificities were also tested in the p83-ELISA.

Nucleotide sequence accession numbers. The nucleotide sequence of the p83 gene of *B. burgdorferi* sensu stricto strain B31 (25) was previously submitted to the EMBL database under accession no. M60802. The sequence of the p83 gene of *B. afzelii* PKo (17) was previously submitted to EMBL under accession no. X71398.

RESULTS

p83-ELISA. On preliminary testing, the best differentiation between positive and negative sera was achieved when the microtiter plates were coated with an antigen concentration of 0.5 μg/ml. When 154 samples from healthy controls were tested, the cutoffs, calculated as the mean extinction plus 2 standard deviations, were 0.20 for IgM and 0.22 for IgG. The reproducibility of ODs from run to run was ±10%. Thus, we defined ranges of borderline results 10% above and below the cutoff: IgM, 0.18 to 0.22; IgG, 0.20 to 0.24.

To test specificity, an additional 82 serum samples from healthy subjects were tested in the p83-ELISA. None had IgG and three (4%) had IgM antibodies (OD values not shown).

Sensitivity (defined as the number of correct positive results divided by the total number of Lyme disease patients) for IgM and IgG antibodies varied, depending on the clinical stage of the illness. IgM or IgG antibodies were detected in 20% (24 of 118: 7 with IgM, 16 with IgG, and 1 with IgM and IgG) of patients with EM, in 42% (14 of 33: 3 with IgM and IgG and 11 with IgG) of patients with NB (all 3 patients with stage III NB had positive results in the p83-ELISA), in 94% (16 of 17: 2 with IgM and IgG and 14 with IgG) of patients with ART, and in 86% (36 of 42: 2 with IgG and IgM and 34 with IgG) of patients with ACA. Borderline values were observed as follows: controls, 3% (5 of 154; 2 IgM and 3 IgG); EM, 6% (7 of 118; 5 IgM and 2 IgG); NB, 3% (1 of 33, IgG), ART, none; and ACA, 5% (2 of 42; both IgG).

Positive reactions with serum samples containing antibodies against *Treponema pallidum* (1 of 37 IgG positive, 1 of 37 borderline) or EBV (1 of 46 IgM positive, none borderline) and with various autoantibodies (2 of 53 IgM positive, 4 of 53 borderline) were rare.

Results of the ELISA are given in detail in Fig. 2. When the

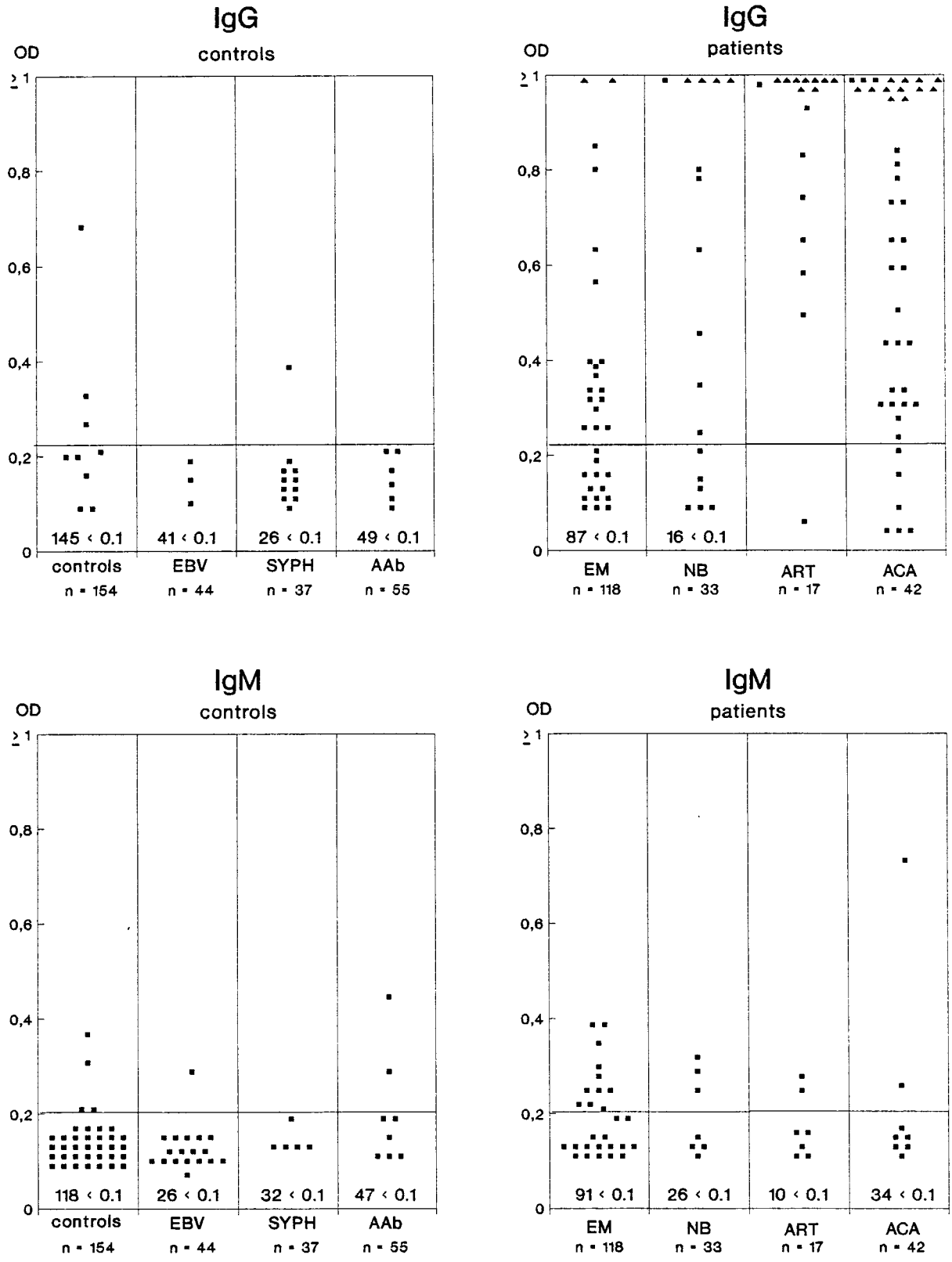

FIG. 2. Results of p83-ELISAs for IgG and IgM antibodies in sera of patients with EM, NB, ART, and ACA. A total of 154 healthy individuals served as controls; patients with EBV infections, syphilis (SYPH), or autoantibodies of various specificities (AAb) were also tested. Horizontal lines mark the diagnostic cutoff level.

reactivities of 36 serum samples from ACA patients and 14 from ART patients with p83 from strain Bo23 and p83 from strain GeHo were compared, concordance was observed.

DISCUSSION

This study presents data from an ELISA employing purified, recombinant p83 from *B. burgdorferi* sensu stricto and *B. afzelii* as an antigen.

In late stages of Lyme borreliosis, the sensitivities were 94% (ART) and 83% (ACA). No difference between results employing recombinant antigens from genomospecies *B. burgdorferi* sensu stricto and *B. afzelii* was found. The sensitivities determined in this study are in accordance with those derived from Western blotting for this antigen by other investigators (7, 8, 22, 35, 36). Dressler et al. (7) performed immunoblots with several *Borrelia* strains, representing the three genomospecies of *B. burgdorferi* (*B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*). They found sensitivities of 80 to 87% and 77 to 81% for ACA and Lyme ART patients, respectively. No significant difference in sensitivity for p93 (the p83 homolog in their study) among the three genomospecies was detected. In contrast, Wilske et al. (32) found 95% (19 of 20) of serum specimens from ACA patients to be positive with recombinant p100 from *B. afzelii* as determined by immunoblotting, whereas only 70% (16 of 20) of the same serum samples were positive with recombinant p100 from *B. burgdorferi* sensu stricto. Our findings are in agreement with those of Dressler et al. (7). We found no relevant serological heterogeneity between the two p83 homologs tested in this study. This reflects the high degree of sequence homology (89%) of the two antigens (17, 28). We expect that there would also be no serological heterogeneity with p83 from genomospecies *B. garinii*, since sequence homology of p83 from this genomospecies to p83 from the other two genomospecies is at least 88% (28). On the basis of our experience, employment of more than one p83 homolog in serological assays should not result in increased sensitivity.

A frequency of 20% (IgG and IgM) for antibodies to p83 in patients with EM lies in the range found by researchers performing Western blotting (31). This indicates that antibodies against p83 are rarely induced in the early stages of infection. Zöller et al. (35) did not detect a 94-kDa band in Western blots until the 6th to 12th week after the presumed onset of infection. An incidence of approximately 42% (IgG and IgM) in patients with NB is also in rough agreement with earlier reports (31).

In this study, the low frequency of IgM antibodies is noteworthy. Only 18 of 210 patients revealed IgM antibodies against p83. Eight of these patients had IgG antibodies as well, indicating a more advanced immune response against spirochetes. Possibly, these are persisting IgM antibodies, as can be observed in other infectious diseases, with a frequency of 5 to 10%. Only 3 of 118 EM patients revealed isolated, clearly positive IgM antibodies against p83. This incidence does not differ significantly from that in the healthy control group, in which only 3 of 82 subjects were positive for IgM antibodies. We conclude that determination of IgM antibodies against p83 is of little diagnostic value in Lyme disease.

For confirmation of specificity, 82 serum samples from healthy controls were tested in addition to the 154 control specimens used to calculate the cutoff. Of the 82 samples, none had IgG and only 3 had IgM antibodies. This reveals high specificity, especially considering that control sera were derived from persons living in areas where Lyme disease is endemic, in which 15 to 20% of ticks are infected with *B. burgdorferi*. A total of 25% of the control group remembered being bitten by a tick at least once within the last 3 years. Equally high specificities for p83 were found by investigators performing Western blots (8, 22, 31, 33, 35, 36).

The incidence of cross-reactivity with autoantibodies (3%) and with antibodies induced by a polyclonal stimulation due to an EBV infection (2%) was low. Thus, a positive titer of antibody to p83 provides strong diagnostic evidence of late-stage Lyme disease. However, whether a positive titer of antibody to p83 correlates with an active Lyme borreliosis infection has yet to be clarified.

Various authors have published serological data for p83 and its homologs obtained by Western blotting; however, there are no reports on the use of recombinant p83 antigen in an ELISA. Compared with the Western blot, an ELISA is a quantitative method whose cutoff can be reliably standardized from a large number of control serum samples. In addition, the ELISA is less expensive and less time-consuming to perform than the Western blot.

Considering the high specificity of the IgG p83-ELISA presented in this study, we conclude that it is a suitable test for serodiagnosis of late-stage Lyme borreliosis.

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REFERENCES

1. **Aguero-Rosenfeld, M. E., J. Nowakowski, D. F. McKenna, C. A. Carbonaro, and G. P. Wormser.** 1993. Serodiagnosis in early Lyme disease. J. Clin. Microbiol. **31:**3090–3095.
2. **Barbour, A. G.** 1988. Laboratory aspects of Lyme borreliosis. Clin. Microbiol. Rev. **1:**399–414.
3. **Barbour, A. G., W. Burgdorfer, S. F. Hayes, O. Peter, and A. Aeschlimann.** 1983. Isolation of a cultivable spirochete from *Ixodes ricinus* ticks in Switzerland. Curr. Microbiol. **8:**123–126.
4. **Bruckbauer, H. R., V. Preac-Mursic, R. Fuchs, and B. Wilske.** 1992. Cross-reactive proteins of *Borrelia burgdorferi*. Eur. J. Clin. Microbiol. Infect. Dis. **11:**224–232.
5. **Coleman, J. L., and J. L. Benach.** 1992. Characterization of antigenic determinants of *Borrelia burgdorferi* shared by other bacteria. J. Infect. Dis. **165:**658–666.
6. **Ditton, H. J., M. Neuss, and L. Zöller.** 1992. Evidence that *Borrelia burgdorferi* immunodominant proteins p100, p94 and p83 are identical. FEMS Microbiol. Lett. **73:**217–220.
7. **Dressler, F., R. Ackermann, and A. C. Steere.** 1994. Antibody responses to the three genomic groups of *Borrelia burgdorferi* in European lyme borreliosis. J. Infect. Dis. **169:**313–318.
8. **Dressler, F., J. A. Whalen, B. N. Reinhardt, and A. C. Steere.** 1993. Western blotting in the serodiagnosis of Lyme disease. J. Infect. Dis. **167:**392–400.
9. **Eiffert, H., T. Schlott, M. Hoppert, H. Lotter, and R. Thommsen.** 1992. Identification of an endoflagellar associated protein in *Borrelia burgdorferi*. J. Med. Microbiol. **36:**209–214.
10. **Feder, H. M., M. A. Gerber, S. W. Luger, and R. W. Ryan.** 1991. False positive serologic tests for Lyme disease after varicella infection. N. Engl. J. Med. **325:**1886–1887. (Letter.)
11. **Fikrig, E., E. D. Hueguenel, R. Berland, D. W. Rahn, J. A. Hardin, and R. A. Flavell.** 1992. Serologic diagnosis of Lyme disease using recombinant outer surface proteins A and B and flagellin. J. Infect. Dis. **165:**1127–1132.
12. **Hansen, K.** 1993. Laboratory diagnostic methods in Lyme borreliosis. Clin. Dermatol. **11:**407–414.
13. **Hansen, K., and E. Åsbrink.** 1989. Serodiagnosis of erythema migrans and acrodermatitis chronica atrophicans by the *Borrelia burgdorferi* flagellum enzyme-linked immunosorbent assay. J. Clin. Microbiol. **27:**545–551.
14. **Hansen, K., P. Hindersson, and N. Strandberg Pedersen.** 1988. Measurement of antibodies to the *Borrelia burgdorferi* flagellum improves serodiagnosis in Lyme disease. J. Clin. Microbiol. **26:**338–346.
15. **Hansen, K., K. Pii, and A.-M. Lebech.** 1991. Improved immunoglobulin M serodiagnosis in Lyme borreliosis by using a μ-capture enzyme-linked immunosorbent assay with biotinylated *Borrelia burgdorferi* flagella. J. Clin. Microbiol. **29:**166–173.
16. **Hofmann, H., and H. R. Bruckbauer.** 1994. Diagnostic value of recombinant protein-immunoblot compared to purified flagellum-ELISA in early Lyme borreliosis, poster PO99T. Presented at the VIth International Conference

on Lyme Borreliosis, Bologna, Italy, 1994.
17. **Jauris-Heipke, S., R. Fuchs, A. Hofmann, F. Lottspeich, V. Preac-Mursic, E. Soutschek, G. Will, and B. Wilske.** 1993. Molecular characterization of the p100 gene of *Borrelia burgdorferi* strain PKo. FEMS Microbiol. Lett. **114:** 235–242.
18. **Kaiser, R.** 1994. Variable CSF findings in early and late Lyme neuroborreliosis: a follow up study in 47 patients. J. Neurol. **246:**26–36.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
20. **Lefebvre, R. B., G.-C. Perng, and R. C. Johnson.** 1990. The 83-kilodalton antigen of *Borrelia burgdorferi* which stimulates immunoglobulin M (IgM) and IgG responses in infected hosts is expressed by a chromosomal gene. J. Clin. Microbiol. **28:**1673–1675.
21. **Luft, B. J., S. Mudri, W. Jiang, R. J. Dattwyler, P. D. Gorevic, T. Fischer, P. Munoz, J. Dunn, and W. H. Schubach.** 1992. The 93-kilodalton protein of *Borrelia burgdorferi*: an immunodominant protoplasmic cylinder antigen. Infect. Immun. **60:**4309–4321.
22. **Ma, B., B. Christen, D. Leung, and C. Vigo-Pelfrey.** 1992. Serodiagnosis of Lyme borreliosis by Western immunoblot: reactivity of various significant antibodies against *Borrelia burgdorferi*. J. Clin. Microbiol. **30:**370–376.
23. **Magnarelli, L. A., J. F. Anderson, and A. G. Barbour.** 1989. Enzyme linked immunosorbent assays for Lyme disease: reactivity of subunits of *Borrelia burgdorferi*. J. Infect. Dis. **159:**43–49.
24. **Magnarelli, L. A., J. N. Miller, J. F. Anderson, and G. R. Riviere.** 1990. Cross-reactivity of nonspecific treponemal antibody in serologic tests for Lyme disease. J. Clin. Microbiol. **28:**1276–1279.
25. **Perng, G.-C., R. B. LeFebvre, and R. C. Johnson.** 1991. Further characterization of a potent immunogen and the chromosomal gene encoding it in the Lyme disease agent, *Borrelia burgdorferi*. Infect. Immun. **59:**2070–2074.
26. **Rasiah, C., S. Rauer, G. S. Gassmann, and A. Vogt.** 1994. Use of a hybrid protein consisting of the variable region of the *Borrelia burgdorferi* flagellin and part of the 83-kilodalton protein as antigen for serodiagnosis of Lyme disease. J. Clin. Microbiol. **32:**1011–1017.
27. **Robinson, J. M., T. J. Pilot-Matias, S. D. Pratt, C. B. Patel, T. S. Bevirt, and J. C. Hunt.** 1993. Analysis of the humoral response to the flagellin protein of *Borrelia burgdorferi*: cloning of regions capable of differentiating Lyme disease from syphilis. J. Clin. Microbiol. **31:**629–635.
28. **Rössler, D., H. Eiffert, S. Jauris-Heipke, G. Lehnert, J. Nossol, V. Preac-Mursic, T. Schlott, E. Soutschek, and B. Wilske.** 1994. Molecular characterization of the P83/100 proteins of various *Borrelia burgdorferi* sensu lato strains, poster P009M. Presented at the VIth International Conference on Lyme Borreliosis, Bologna, Italy, 1994.
29. **Stiernstedt, G., R. Dattwyler, P. H. Duray, K. Hansen, J. Jirous, R. C. Johnson, M. Karlsson, V. Preac-Mursic, and T. G. Schwan.** 1991. Diagnostic tests in Lyme borreliosis. Scand. J. Infect. Dis. **77:**136–142.
30. **Volkman, D. J., B. J. Luft, P. D. Gorevic, J. Shultz, and L. Padovano.** 1991. Characterization of an immunoreactive 93-kDa core protein of *Borrelia burgdorferi* with a human IgG monoclonal antibody. J. Immunol. **146:**3177–3182.
31. **Wilske, B., V. Fingerle, P. Herzer, A. Hofmann, G. Lehnert, H. Peters, H.-W. Pfister, V. Preac-Mursic, E. Soutschek, and K. Weber.** 1993. Recombinant immunoblot in the serodiagnosis of Lyme borreliosis. Comparison with indirect immunofluorescence and enzyme-linked immunosorbent assay. Med. Microbiol. Immunol. **182:**255–270.
32. **Wilske, B., V. Fingerle, V. Preac-Mursic, S. Jauris-Heipke, A. Hofmann, H. Loy, H.-W. Pfister, D. Rössler, and E. Soutschek.** 1994. Immunoblot using recombinant antigens derived from different genospecies of *Borrelia burgdorferi* sensu lato. Med. Microbiol. Immunol. **183:**43–59.
33. **Wilske, B., V. Preac-Mursic, R. Fuchs, H. Bruckbauer, A. Hofmann, G. Zumstein, S. Jauris, E. Soutschek, and M. Motz.** 1990. Immunodominant proteins of *Borrelia burgdorferi*: implications for improving serodiagnosis of Lyme borreliosis, p. 47–63. *In* H. C. Neu (ed.), New antibacterial strategies. Churchill Livingstone, London.
34. **Wilske, B., V. Preac-Mursic, G. Liegl, and W. Gueyl.** 1989. Detection of IgM and IgG antibodies to *Borrelia burgdorferi* using different strains as antigens. Zentralbl. Bakteriol. Suppl. **18:**299–309.
35. **Zöller, L., S. Burkhard, and H. Schäfer.** 1991. Validity of Western immunoblot band patterns in the serodiagnosis of Lyme borreliosis. J. Clin. Microbiol. **29:**174–182.
36. **Zöller, L., J. Cremer, and M. Faulde.** 1993. Western blot as a tool in the diagnosis of Lyme borreliosis. Electrophoresis **14:**937–944.