

Serodiagnosis of Erythema Migrans and Acrodermatitis Chronica Atrophicans by the *Borrelia burgdorferi* Flagellum Enzyme-Linked Immunosorbent Assay

KLAUS HANSEN^{1*} AND EVA ÅSBRINK²

Borrelia Laboratory, Department of Treponematoses, Statens Seruminstitut, 2300 Copenhagen S., Denmark,¹ and Department of Dermatology, Södersjukhuset, Karolinska Institut, Stockholm, Sweden²

Received 15 August 1988/Accepted 28 November 1988

The diagnostic performance of an enzyme-linked immunosorbent assay (ELISA) using purified *Borrelia burgdorferi* flagella as test antigen was compared with that of a *B. burgdorferi* sonic extract ELISA. We tested sera from 200 healthy controls, 107 patients with erythema migrans (EM), 50 patients with acrodermatitis chronica atrophicans (ACA), and 98 patients with various dermatological disorders without clinical evidence of active Lyme borreliosis. The flagellum ELISA was significantly more sensitive than the sonic extract ELISA. With sera from patients with EM, the diagnostic sensitivity for immunoglobulin G (IgG) antibody detection increased from 11.2 to 35.5% ($P < 0.001$) and for IgM antibody detection it increased from 16.6 to 44.8% ($P < 0.001$). In the flagellum ELISA, the number of positive tests increased significantly ($P < 0.005$) when the duration of EM exceeded 1 month, but still only about 50% of patients with longstanding (1 to 12 months) untreated EM were IgG seropositive. Concomitant general symptoms did not affect the antibody level, whereas patients with multiple erythema were more frequently seropositive. All sera from patients with EM which were positive in the sonic extract ELISA were also positive in the flagellum ELISA. Not only did the overall number of positive tests increase, but the flagellum ELISA yielded a significantly better quantitative discrimination between seropositive patients and controls ($P < 0.002$). IgG antibodies to the *B. burgdorferi* flagellum were found in all sera from patients with ACA, indicating persistence of an anti-flagellum immune response in late stages of Lyme borreliosis. IgM reactivity in sera from patients with ACA was shown to be unspecific and the result of IgM rheumatoid factor. A rheumatoid factor was detected in sera from 32% of patients with ACA, compared with 7.5% of patients with EM. The improved diagnostic performance, the ease of standardization of the flagellum antigen, and the lack of strain variation make the *B. burgdorferi* flagellum a needed reference antigen for growing routine serology in Lyme borreliosis.

Serological tests for antibodies to *Borrelia burgdorferi* have been available since 1982 (9). The presently used tests, indirect immunofluorescence assay and enzyme-linked immunosorbent assay (ELISA), use whole cells or whole-cell sonic extracts as antigen. These tests are of limited diagnostic value in early disease, since they yield low diagnostic sensitivities. Only 10 to 40% of patients with erythema migrans (EM) are reported to be seropositive (1, 3, 5, 10, 20-22, 26). A low antigen load, a late and slow humoral immune response, and the quality of the test antigen used may be responsible. *B. burgdorferi* shares immunogenic antigens with many other bacteria, not only spirochetes (7, 14). The inclusion of such cross-reactive antigens in the test antigen may be responsible for the low diagnostic specificity of the presently used tests. A single, immunodominant, and more specific *Borrelia* antigen should be used for a serodiagnostic assay. Several Western (immuno-) blotting (WB) studies have shown that the human immune response in Lyme borreliosis early and constantly recognizes the 41-kilodalton band corresponding to the flagellum (7, 11-13, 17, 25). In a recent study, we showed that the use of purified *B. burgdorferi* flagellum as ELISA antigen significantly improved immunoglobulin G (IgG) and IgM serodiagnosis, especially in early cases of lymphocytic meningoradiculitis (Bannwarth's syndrome) (15).

The aim of the present study was to investigate the diagnostic efficiency of the *B. burgdorferi* flagellum ELISA

in patients with EM, the primary stage of Lyme borreliosis, and in patients with the late dermatological manifestation of *B. burgdorferi* infection, acrodermatitis chronica atrophicans (ACA), which may start 0.5 to 8 years after EM (2).

MATERIALS AND METHODS

Patients. A total of 107 patients presenting with a typical, clinically uncomplicated EM entered the study. There were 26 males and 81 females. They were aged 6 to 83 years (median age, 54 years). Ninety-one patients had a solitary EM; sixteen experienced multiple erythema. Thirty-eight patients reported accompanying constitutional symptoms (low-grade fever, headache, musculoskeletal pain), and sixty-nine had only the skin lesion. The time from onset of the EM until blood sampling took place ranged from 2 days to 12 months, with a median duration of 3.5 weeks.

Another 50 patients with ACA, including 16 males and 34 females, were investigated. They were aged 28 to 89 years (median age, 61 years). The duration of the ACA in these patients ranged from 0.5 to 20 years (median duration, 2.5 years).

The diagnosis of EM and ACA was based on clinical evidence and in every case was made by one of us (E.Å.). The clinical diagnosis of ACA was confirmed by histopathology (2). All the patients were from the Stockholm area and were seen between 1984 and 1987. All serological measurements were done on pretreatment sample 1.

Controls. Sera from 200 healthy Danish controls were used for determination of the 95% specific cutoff level in both

* Corresponding author.

tests. The Swedish control group consisted of 98 patients with various dermatological disorders without clinical evidence of an active *B. burgdorferi* infection. This was to assure the applicability of the cutoff level defined by the Danish controls to the Swedish patient population. Sera from patients and controls were stored at -20°C until use.

***B. burgdorferi* test antigens.** A Swedish *B. burgdorferi* strain, ACA-1, isolated from the skin of a patient with ACA (4) was used for all antigen preparations. Spirochetes were grown for 5 days in BSK II medium (6) at 32°C to a cell density of $10^8/\text{ml}$. The sonic extract antigen for ELISA was prepared as described previously (15). The spirochetes were washed three times in phosphate-buffered saline (PBS; pH 7.4) and sonicated on ice by seven 15-s blasts with an MSE 150 W ultrasonic disintegrator (Manor Royal, Crawley, England). The sonic extract was centrifuged ($10,000 \times g$, 30 min), and the supernatant primarily containing the soluble antigens was used for ELISA. This sonic extract contained flagellar components according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and WB examination.

Isolation of *B. burgdorferi* flagellum. The purification procedure for the *B. burgdorferi* flagellum was recently reported in detail (15). Briefly, the spirochetes were subjected to a mild-ionic detergent for removal of the outer envelope. The detergent-insoluble material containing the protoplasmic cylinders with the periplasmic flagella attached was sheared in a blender to achieve a mechanical detachment of the flagella from the cell bodies. The sheared material was then subjected to several differential centrifugations. The final and most important step in the purification was a banding on a CsCl density gradient. Visible bands were isolated separately and examined by WB to assess the yield and purity of the 41-kilodalton flagellum antigen. Flagella containing bands were pooled and dialyzed against PBS. The *B. burgdorferi* flagellum antigen prepared in this way proved to be highly pure by electron microscopy, WB, and crossed immunoelectrophoresis, as shown previously (15).

ELISA procedure. The *B. burgdorferi* sonic extract ELISA and the flagellum ELISA were performed identically except for the antigen. Flat-bottomed polystyrene microwell plates (Immunoplates, code 2-69620; Nunc, Roskilde, Denmark) were coated overnight at 4°C with $100 \mu\text{l}$ of antigen diluted in PBS. The optimal coating concentration was defined as the antigen dilution resulting in the highest ratio of the optical densities (ODs) between positive and negative control sera. Unspecific protein binding was blocked with 1% (wt/vol) bovine serum albumin in PBS. The wells were washed, and $100 \mu\text{l}$ of serum diluted 1:200 in PBS with 0.5% (wt/vol) bovine serum albumin and 0.05% (vol/vol) Tween 20 was added to the wells and incubated for 2 h at 20°C . After washing, $100 \mu\text{l}$ of peroxidase conjugate was added, either rabbit anti-human IgG or anti-human IgM (codes P-214 and P-215; Dakopats, Copenhagen, Denmark) diluted 1:10,000 and 1:1,000, respectively, in PBS with 0.05% (vol/vol) Tween 20. After incubation for 2 h at 20°C , the plates were washed, and $200 \mu\text{l}$ of the substrate *o*-phenylenediamine (0.41 mg/ml; Sigma Chemical Co., St. Louis, Mo.) in citrate buffer (pH 5) with 0.04% (vol/vol) H_2O_2 was added to each well. After 15 min under protection from light, the enzymatic reaction was stopped by the addition of $50 \mu\text{l}$ of 3 M H_2SO_4 . The OD at 492 nm was read by a colorimeter (Immuno Reader NJ 2000; Nippon, InterMed, Tokyo, Japan). All washings were done three times with 0.56% (wt/vol) NaCl containing 0.05% (vol/vol) Tween 20. Positive and negative control sera were included on every plate. Samples were tested in duplicate, and the mean value was calculated. If the

two values differed more than 10% from the mean, the sample was retested. To eliminate plate-to-plate and day-to-day variations, samples of three serum pools with known low, medium, and high titers were included on every plate for construction of a standard curve. The OD value of every sample was adjusted to this standard curve.

Measurement and absorption of IgM RF. All samples from patients with EM and ACA were investigated for IgM rheumatoid factor (RF). An ELISA with human IgG was used, and the results were evaluated according to the 95% specific upper limit of the assay, 8 IU/ml (16). To eliminate unspecific IgM reactivity resulting from IgM RF, all IgM-reactive samples from patients with ACA were subjected to IgM RF absorption with a commercially available kit (RF Absorbans; Behringwerke, Marburg, Federal Republic of Germany).

Statistical analysis. The diagnostic sensitivities of the sonic extract and flagellum ELISAs were compared by using McNemar's test, assuming a binominal distribution of paired data. Nonpaired data were compared by using the chi-square test. Furthermore, the precise quantitative discrimination of the two assays between controls and seropositive patients was estimated. For each individual sample that was positive in both ELISAs, the distance from the achieved OD value to the cutoff level was calculated for both assays. These differences were compared by using Wilcoxon's rank sum test for paired data.

RESULTS

Measurement of IgG antibodies to the flagellum of *B. burgdorferi* in the sera of 200 healthy controls demonstrated a significant increase in specificity compared with IgG measurement with a sonic extract ELISA (Fig. 1). Using a 95% specific upper limit in both tests, the diagnostic cutoff level could be lowered from 0.400 to 0.160 OD value in the flagellum assay. For IgM antibodies in healthy controls, there was no significant increase in specificity, since the 95% specific cutoff levels were 0.260 and 0.230 OD values, respectively (Fig. 2).

The investigation of the Swedish control group consisting of patients with various dermatological disorders assured that the diagnostic 95% specific cutoff level in both assays based on the Danish control population was also representative and suitable when Swedish patients were studied (Fig. 1 and 2).

When IgG and IgM antibodies in sera from 107 EM patients were measured, the flagellum ELISA showed overall increases in diagnostic sensitivity from 11.2 to 35.5% ($P < 0.001$) for IgG and from 16.6 to 44.8% ($P < 0.001$) for IgM detection compared with the sonic extract ELISA (Table 1). A separate evaluation of the 70 patients with an EM duration of less than 1 month and the 37 patients with an EM duration of more than 1 month revealed similar significant increases in diagnostic sensitivity of the IgG and IgM flagellum ELISA ($P < 0.001$) at both times. Not only did the overall number of positive results increase significantly, but so did the quantitative discrimination between controls and patients. This was the result of a generally higher signal obtained in the flagellum ELISA in addition to the lower cutoff level (Fig. 3a and b).

All sera that were reactive in the sonic extract ELISA were also reactive in the flagellum ELISA (Fig. 3). The sera of 69 patients (64.5%) were either IgG or IgM reactive in the flagellum ELISA, compared with the sera of 28 patients (26.2%) in the sonic extract ELISA. If the assay sensitivity

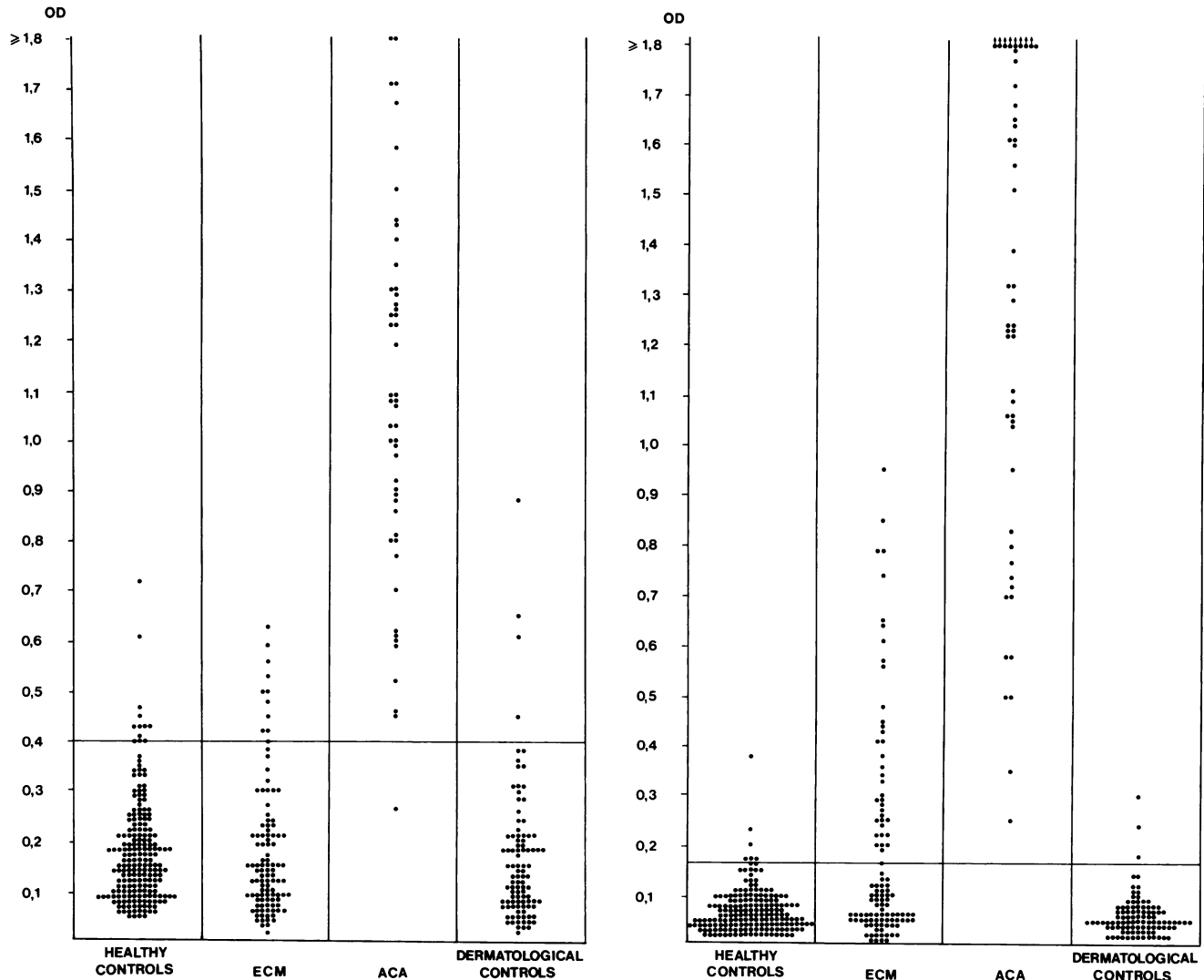


FIG. 1. IgG levels measured by *B. burgdorferi* sonic extract ELISA (left) and *B. burgdorferi* flagellum ELISA (right) in sera of 107 patients with EM (ECM) and 50 patients with ACA. Serving as controls were 200 healthy individuals and 98 patients with various dermatological disorders. The horizontal lines mark the diagnostic 95% specific cutoff levels.

is expressed as a combined evaluation of IgG and IgM data, e.g., either IgG or IgM reactivity, it must be remembered that the diagnostic specificity will decrease to about 90%, since 10% of all controls were either IgG or IgM positive. The sera of 15 patients (14.0%) were both IgG and IgM reactive in the flagellum ELISA, compared with the sera of only 2 patients (1.9%) in the sonic extract ELISA. None of the 298 control individuals was IgG and IgM seroreactive.

When the 107 patients with EM were divided into four groups according to the duration of the erythema, the frequency of seropositive samples increased after a disease duration of more than 1 month (Table 1). This increase was statistically significant only in the IgG flagellum ELISA ($P < 0.005$).

There were 16 patients with multiple erythema. When the serological findings for these patients were compared with those for patients with a solitary EM, an increased IgM reactivity was shown (68.8 versus 39.5%) ($P < 0.05$) (Table 2). Three patients with multiple erythema had a disease duration of more than 2 months; they were all IgG seroreactive. There was no correlation of the specific antibody

level with the occurrence of general symptoms concomitant to the EM (Table 2).

Fifty serum specimens from patients with ACA were tested. For IgG antibodies, the diagnostic sensitivities of the sonic extract and flagellum ELISA were identical, 98 and 100%, respectively. As in EM, the individual OD signal obtained was significantly higher in the IgG flagellum ELISA (Fig. 3c). The number of IgM-seroreactive patients with ACA was reduced from 22 to 12% by the flagellum ELISA.

To rule out the possible role of an IgM RF as the cause of unspecific IgM reactivity, all sera from patients with EM and ACA were investigated for IgM RF. A total of 16 (32%) of 50 patients with ACA and 8 (7.5%) of 107 patients with EM had IgM RFs in the ranges of 10 to 150 and 10 to 35 IU/ml, respectively. Nine of eleven patients with ACA whose sera were IgM reactive in the sonic extract ELISA had detectable IgM RF, including all sera with OD values of >0.3 . The sera of all six patients with ACA that were IgM reactive in the flagellum ELISA had an IgM RF. Absorption of the RF abolished IgM reactivity in all IgM-reactive samples from patients with ACA in both assays. Since only 3 of 48

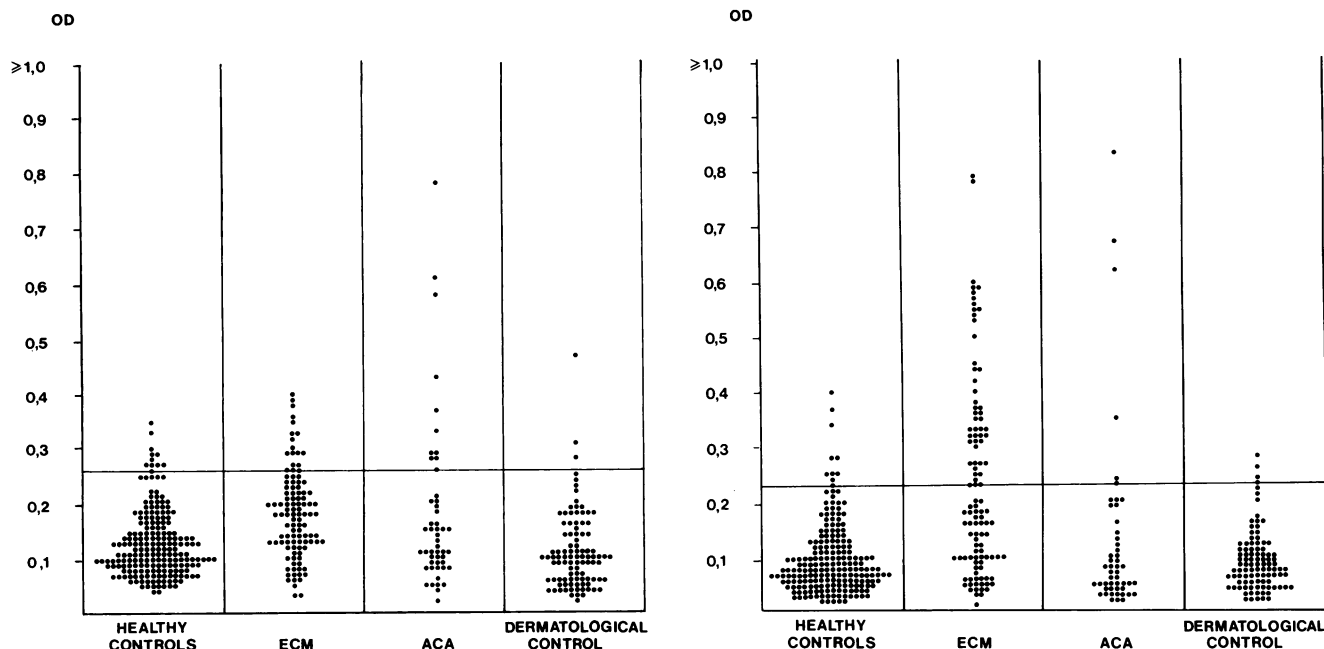


FIG. 2. IgM levels measured by *B. burgdorferi* sonic extract ELISA (left) and *B. burgdorferi* flagellum ELISA (right) in sera of 107 patients with EM (ECM) and 50 patients with ACA. Serving as controls were 200 healthy individuals and 98 patients with various dermatological disorders. The horizontal lines mark the diagnostic 95% specific cutoff levels.

IgM-reactive serum specimens from patients with EM showed an IgM RF, no absorption of the RF was done with these specimens.

DISCUSSION

In a previous study, the *B. burgdorferi* flagellum ELISA was shown to be clearly superior to a sonic extract ELISA in testing serum and cerebrospinal fluid samples from patients with lymphocytic meningoradiculitis (15). A comparable increase in the diagnostic performance of the flagellum ELISA was found in the present investigation of sera from 107 patients with EM, the early and first-stage manifestation of Lyme borreliosis.

A main advantage of the purified antigen was a greatly increased specificity of the IgG ELISA, since the OD values in the control groups were significantly lower. When the diagnostic cutoff level was adjusted to be 95% specific, the gained specificity was converted into a marked increase in diagnostic sensitivity. The diagnostic specificity of the IgM flagellum ELISA was almost unaltered, whereas the diagnostic sensitivity increased significantly.

TABLE 1. Diagnostic sensitivity of *B. burgdorferi* sonic extract and flagellum ELISAs

Wk after onset of EM (no. of patients)	No. (%) of positive samples			
	Sonic extract ELISA		Flagellum ELISA	
	IgG	IgM	IgG	IgM
≤1 (22)	2 (9.1)	2 (9.1)	6 (27.2)	7 (31.8)
2-4 (48)	3 (6.3)	11 (22.9)	12 (25.0)	22 (45.8)
5-12 (32)	6 (18.8)	5 (15.6)	17 (53.1)	17 (53.1)
>12 (5)	1 (20.0)		3 (60.0)	2 (40.0)
Total (107)	12 (11.2)	18 (16.6)	38 (35.5)	48 (44.8)

The improved diagnostic performance of the flagellum ELISA compared with the sonic extract ELISA is most likely the result of the early and strong antibody response to the 41-kilodalton antigen recognized in several WB studies (7, 11-13, 17, 25) and the elimination of irrelevant cross-reacting antigens which are contained in the whole-cell sonic extract. *B. burgdorferi* shares antigenic epitopes with other spirochetes (7, 15, 18) and (and this may be of more practical importance to serology) also with many remotely related and common bacteria, including the normal human flora. Such an antigen is the immunogenic 60-kilodalton protein of *B. burgdorferi* which recently was shown to be the widely cross-reacting common antigen (14). This protein is soluble and is present in every sonic extract of *B. burgdorferi*.

A recent ELISA study (13) evaluated a sonic extract antigen versus a flagellum-enriched but not purified flagellum antigen preparation for ELISA. Possibly because of many residual antigens in the antigen preparation, no significant increase in the number of seropositive patients was found. In agreement with two previous studies (11, 15), Grodzicki and Steere (13) noticed a clearly improved discrimination between controls and patients, since positive sera generally gave considerably higher OD signals when either the flagellum-enriched or purified flagellum antigen was used. This observation is of great importance, especially in growing routine serology, since borderline values in sonic extract ELISAs are frequent and difficult or impossible to interpret.

In patients with a disseminated infection, as in lymphocytic meningoradiculitis, the magnitude of the antibody response to *B. burgdorferi* is correlated with the duration of the disease (12, 15, 21, 23). IgM titers show a maximum at 5 to 7 weeks after onset, whereas IgG titers increase gradually and are positive in almost every untreated case after 2 months (15). In EM, only the use of the flagellum ELISA showed a significant increase ($P < 0.005$) in the number of IgG-positive tests when the duration of an untreated EM

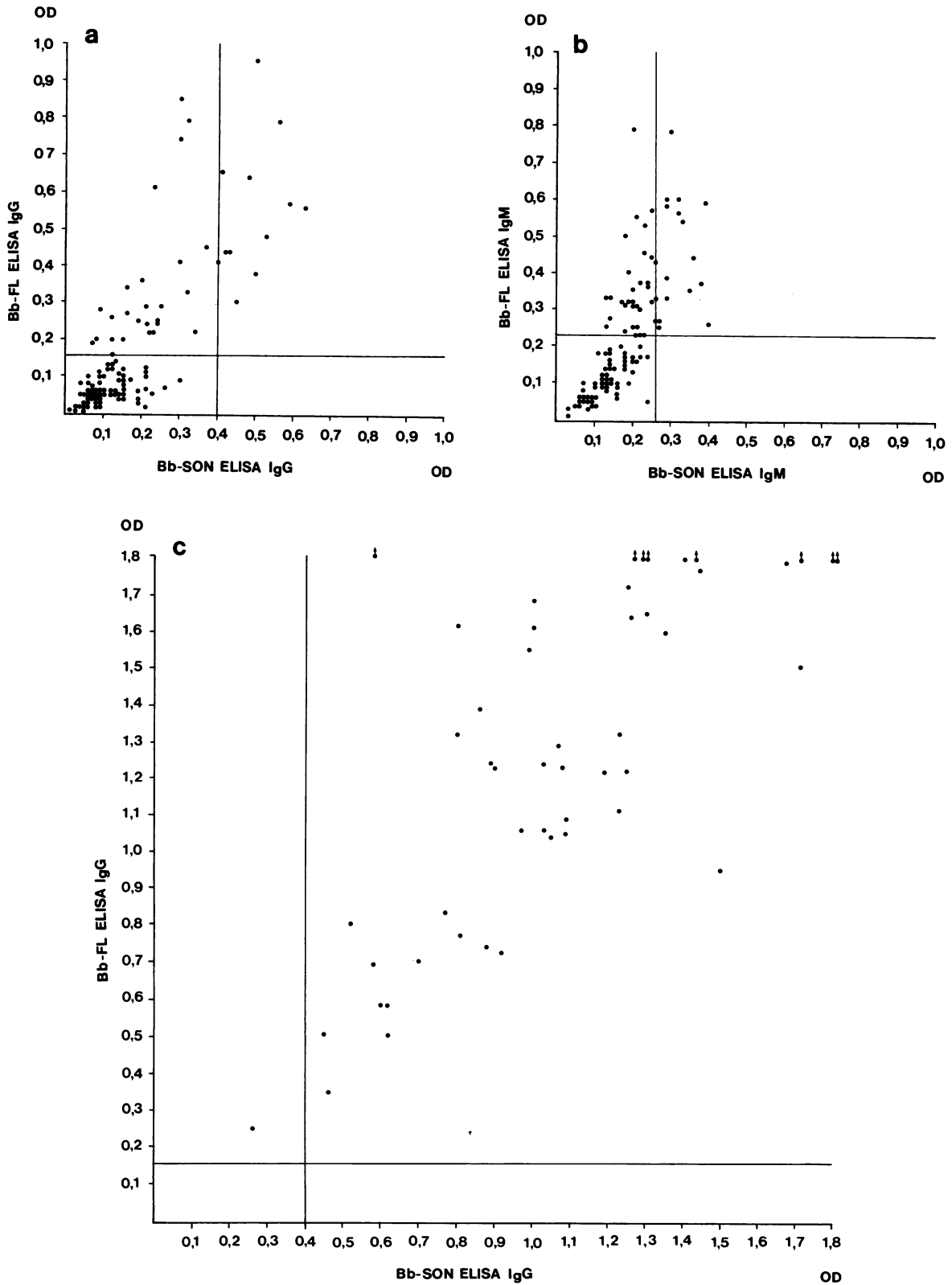


FIG. 3. Correlation of IgG (a) and IgM (b) measurement in sera of 107 patients with EM and of IgG measurement in sera of 50 patients with ACA (c) by the *B. burgdorferi* sonic extract and flagellum ELISAs. The vertical lines represent the cutoff levels in the sonic extract ELISA, and the horizontal lines mark the cutoff levels in the flagellum ELISA. The flagellum ELISA improved the quantitative discrimination between controls and seropositive samples significantly, as estimated by comparing the distances of the achieved OD values from the cutoff level in each test using Wilcoxon's rank sum test for paired data: panel a, $P = 0.002$; panel b, $P = 0.001$; panel c, $P = 0.001$.

TABLE 2. Diagnostic sensitivity of *B. burgdorferi* flagellum ELISA for 107 patients with EM

Positive reaction	% of patients				Avg (n = 107)
	Multiple erythema (n = 16)	Solitary erythema (n = 91)	Concomitant general symptoms (n = 38)	No concomitant general symptoms (n = 69)	
IgM	68.8	39.5	44.7	43.5	44.8
IgG	37.5	36.3	39.5	34.8	35.5

exceeded 1 month. Still, only slightly more than 50% of the patients with longstanding EM (1 to 12 months) were IgG seropositive (Table 1). The reason for this discrepancy is unknown. It may be because uncomplicated EM is an infection restricted to the skin, whereas a disseminated infection leads to a more predictable and stronger immune response. This explanation is supported by the observation of higher antibody levels in patients with multiple skin lesions, which are most likely the result of hematogenous spread. The restriction of a *B. burgdorferi* infection to a solitary skin lesion may be controlled by host factors and properties of the spirochete that determine its pathogenicity. The spontaneous course of the infection, as well as the production of antibodies, seems to be a question not only of disease duration. Therefore, the diagnostic sensitivity of serological tests must be defined for cases of EM and separately for cases of systemic *Borrelia* infection and not for cases of unspecified Lyme borreliosis.

Many serological studies using indirect immunofluorescence assays or sonic extract ELISAs have been reported since 1982. The diagnostic sensitivities achieved vary considerably. The results are often not comparable for several reasons: (i) unequal selection or lack of classification of patients according to the type of manifestation and disease duration; (ii) combined evaluation of IgM and IgG results, e.g., determining the number of patients who are either IgM or IgG seropositive without noting that this procedure reduces the diagnostic specificity significantly; expression of the diagnostic sensitivity of a test as the rate of either IgG or IgM reactivity demands at least a 97.5% specific cutoff level in both the IgG and IgM assays; (iii) inclusion of several samples from one patient or consideration of only the highest titer measured in a patient and not the first, diagnostic, pretreatment sample; (iv) different criteria for size and composition of the control group; and (v) very different cutoff levels. In a diagnostic or screening test, the cutoff level should be at least 95% specific based on a large and not serologically preselected control group.

There were no differences regarding the IgM or IgG antibody levels in 200 Danish and 98 Swedish controls representing two independent populations from distant regions. Such differences could have been the result of regional differences in tick or *B. burgdorferi* exposure of the populations or differences in the regional prevalences of certain strains. Previous indirect immunofluorescence assay studies that tested sera with American and European *B. burgdorferi* strains did not show any strain-dependent differences (1, 3, 19, 26). The use of *B. burgdorferi* flagellum as test antigen will further diminish the possible influence of strain variation on serological results, since it is a genuswide antigen (8).

IgG antibodies to the *B. burgdorferi* flagellum were found in all sera from patients with ACA. This proves the persis-

tence of an antibody response to the flagellum even into the late stages of the disease.

IgM RF may cause false-positive IgM reactivity in an indirect ELISA (24). Therefore, samples from patients with EM and ACA were investigated for an IgM RF. Of the samples from patients with ACA, 32% had a detectable IgM RF, including 9 of 11 serum samples that were reactive in the sonic extract ELISA for IgM and 6 of 6 samples that were reactive in the flagellum ELISA for IgM. In accordance with a previous study (26), pretreatment of the sera with IgM RF Absorbans abolished the IgM reactivity in all IgM-reactive samples from patients with ACA. False IgM reactivity caused by IgM RF in EM is probably rare, since only 3 of 48 IgM-reactive serum samples had a demonstrable RF. IgM RF is frequently found in various acute and chronic infectious and immunological disorders. For routine serology, we therefore recommend that the use of the indirect IgM ELISA be restricted to probable early cases of Lyme disease and, in particular, to the investigation of cerebrospinal fluid (15).

The *B. burgdorferi* flagellin (the reduced protein subunit of the flagellum antigen) has genus-specific epitopes (8) but also some more conserved epitopes cross-reacting with other spirochetal flagellins, especially from treponemas. Therefore, the main limitation of the diagnostic specificity of the *B. burgdorferi* flagellum as a test antigen is a partial cross-reactivity with the *Treponema pallidum* flagellum (15). The new test does not permit serological discrimination between patients with Lyme borreliosis and syphilis, although serological cross-reactivity was reduced compared with that of the sonic extract ELISA (15). The magnitude of this problem in routine serology is limited. During one year, 1987, when a sonic extract ELISA was still being run, the Copenhagen Borrelia Laboratory received 5,264 samples. Only four were false-positive because of syphilitic infections. Furthermore, patients with Lyme borreliosis and syphilis are easily differentiated clinically and serologically by the nontreponemal syphilis serological tests (19).

On the basis of the present study of sera of patients with EM and ACA and a previous investigation of sera and cerebrospinal fluid of patients with lymphocytic meningoradiculitis (15), we conclude that the *B. burgdorferi* flagellum ELISA is for the time being the most sensitive and specific quantitative serological test. WB does not seem to be a practicable method for routine serology. Neither a single *B. burgdorferi*-specific band nor a combination of such bands with a high diagnostic sensitivity that would allow the discrimination of specific and unspecific antibody reactivities has been identified (13, 17). The flagellum ELISA is of particular value when the specific antibody level is low, as in many cases of EM and in the early secondary stage of Lyme borreliosis (15). A low but specific antibody response is often missed by a sonic extract ELISA because it is hidden by the necessarily high cutoff level. The antigenic composition of a whole-cell sonic extract may vary considerably, depending on the strain and the preparation technique. The *B. burgdorferi* flagellum is easy to standardize as a test antigen. These properties make the *B. burgdorferi* flagellum a suitable and needed reference antigen for routine serodiagnosis in Lyme borreliosis.

ACKNOWLEDGMENTS

Klaus Hansen was supported by a grant from the University of Copenhagen and the Mauritzen la Fontaine foundation. Eva Åsbrink received a grant (no. 7935) from the Swedish Medical Research Council.

We thank Hanna Hansen for perfect technical assistance; Karen Langner for preparing the manuscript; Kirsten Christoffersen, Department of Biostatistics, Statens Seruminstitut, for statistical calculations; and Mimi Høier-Madsen, Department of Clinical Immunology, Statens Seruminstitut, for measuring IgM RF.

LITERATURE CITED

- Ackermann, R., J. Kabatzki, H. P. Boisten, A. C. Steere, R. L. Grodzicki, S. Hartung, and U. Runne. 1984. Spirochaeten Aetiologie der Erythema chronicum migrans Krankheit. Dtsch. Med. Wochenschr. **109**:92-97.
- Åsbrink, E., A. Brehmer-Andersson, and A. Hovmark. 1986. Acrodermatitis chronica atrophicans—a spirochetosis. Am. J. Dermatol. **8**:209-219.
- Åsbrink, E., B. Hederstedt, and A. Hovmark. 1984. The spirochetal etiology of erythema chronicum migrans Afzelius. Acta Dermato-Venereol. **64**:291-295.
- Åsbrink, E., A. Hovmark, and B. Hederstedt. 1984. The spirochetal etiology of acrodermatitis chronica atrophicans Herxheimer. Acta Dermato-Venereol. **64**:506-512.
- Åsbrink, E., A. Hovmark, and B. Hederstedt. 1985. Serologic studies of erythema chronicum migrans Afzelius and acrodermatitis chronica atrophicans with indirect immunofluorescence and enzyme-linked immunosorbent assays. Acta Dermato-Venereol. **65**:509-514.
- Barbour, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57**:521-525.
- Barbour, A. G., W. Burgdorfer, E. Grunwaldt, and A. C. Steere. 1983. Antibodies of patients with Lyme disease to components of the Ixodes dammini spirochete. J. Clin. Invest. **72**:504-515.
- Barbour, A. G., S. F. Hayes, R. A. Heiland, M. E. Schrupf, and S. L. Tessier. 1986. A *Borrelia*-specific monoclonal antibody binds to a flagellar epitope. Infect. Immun. **52**:549-554.
- Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davies. 1982. Lyme disease—a tick-borne spirochetosis? Science **216**:1317-1319.
- Centers for Disease Control. 1988. Lyme disease—Connecticut. Morbid. Mortal. Weekly Rep. **37**:1-3.
- Coleman, J. L., and J. L. Benach. 1987. Isolation of antigenic components from Lyme disease spirochete: their role in early diagnosis. J. Infect. Dis. **155**:756-765.
- Craft, J. E., D. K. Fisher, G. T. Shimanto, and A. C. Steere. 1986. Antigens of *Borrelia burgdorferi* recognized during Lyme disease. J. Clin. Invest. **78**:934-939.
- Grodzicki, R. L., and A. C. Steere. 1988. Comparison of immunoblotting and indirect ELISA using different antigen preparations for diagnosing early Lyme disease. J. Infect. Dis. **157**:790-797.
- Hansen, K., J. M. Bangsborg, H. Fjordvang, N. S. Pedersen, and P. Hinderesson. 1988. Immunochemical characterization of and isolation of the gene for a *Borrelia burgdorferi* immunodominant 60-kilodalton antigen common to a wide range of bacteria. Infect. Immun. **56**:2047-2053.
- Hansen, K., P. Hinderesson, and N. S. Pedersen. 1988. Measurement of antibodies to the *Borrelia burgdorferi* flagellum improves serodiagnosis in Lyme disease. J. Clin. Microbiol. **26**:338-346.
- Høier-Madsen, M., L. P. Nielsen, and S. Møller. 1986. Determination of IgM rheumatoid factors by enzyme-linked immunosorbent assay (ELISA). Ugeskr. Laeg. **148**:2018-2021.
- Karlson, M., I. Møllegaard, G. Stiernstedt, A. M. Henrikson, and B. Wretling. 1988. Characterization of antibody response in patients with *Borrelia meningitis*. Serodiagnosis Immunother. **2**:375-386.
- Magnarelli, L. A., J. F. Anderson, and R. C. Johnson. 1987. Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. J. Infect. Dis. **156**:183-188.
- Russel, H., J. S. Sampson, G. P. Schmid, H. W. Wilkinson, and B. Plikaytis. 1984. Enzyme-linked immunosorbent assay and indirect immunofluorescence assay for Lyme disease. J. Infect. Dis. **149**:465-470.
- Shrestha, M., R. L. Grodzicki, and A. C. Steere. 1985. Diagnosing early Lyme disease. Am. J. Med. **78**:235-240.
- Steere, A. C., R. L. Grodzicki, A. N. Kornblatt, J. E. Craft, A. G. Barbour, W. Burgdorfer, G. P. Schmid, E. Johnson, and S. E. Malawista. 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308**:733-740.
- Stiernstedt, G. T., G. Eriksson, W. Enfors, H. Jörbeck, B. Svenningsson, B. Sköldenberg, and M. Granström. 1986. Erythema chronicum migrans in Sweden: clinical manifestations and antibodies to *Ixodes ricinus* spirochete measured by indirect immunofluorescence and enzyme-linked immunosorbent assay. Scand. J. Infect. Dis. **18**:217-224.
- Stiernstedt, G. T., M. Granström, B. Hederstedt, and B. Sköldenberg. 1985. Diagnosis of spirochetal meningitis by enzyme-linked immunosorbent assay and indirect immunofluorescence assay in serum and cerebrospinal fluid. J. Clin. Microbiol. **21**:819-825.
- Vejtorp, M. 1980. The interference of IgM rheumatoid factor in enzyme-linked immunosorbent assays of rubella IgM and IgG antibodies. J. Virol. Methods **1**:1-9.
- Wilske, B., V. Preac-Mursic, G. Schierz, and K. V. Busch. 1986. Immunochemical and immunological analysis of European *Borrelia burgdorferi* strains. Zentralbl. Bakteriell. Hyg. A **263**:92-102.
- Wilske, B., G. Schierz, V. Preac-Mursic, K. Weber, H. W. Pfister, and V. K. Einhäupl. 1984. Serological diagnosis of erythema chronicum migrans disease and related disorders. Infection **12**:331-337.