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ORIGINAL INVESTIGATION

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Analysis of the human antibody response to outer surface protein C (OspC) of *Borrelia burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*

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Abstract The aim of this study was to determine by Western blotting (WB) the prevalence of anti-outer surface protein C (OspC) IgM and IgG antibodies in patients with Lyme borreliosis according to each of the three genospecies of *Borrelia burgdorferi* sensu lato. Strains of *B. burgdorferi* sensu stricto (MUL), *B. garinii* (DK 6), and *B. afzelii* (DK 26) served as antigen, all of which expressed abundant OspC. We examined sera from 117 patients with untreated early and late Lyme borreliosis, as well as from 100 blood donors and 29 patients with syphilis. WB results were compared with the *B. burgdorferi* flagellum enzyme-linked immunosorbent assay (ELISA) data. OspC from *B. burgdorferi* sensu stricto showed the lowest diagnostic sensitivity. OspC from *B. garinii* and *B. afzelii* performed almost identically in erythema migrans, with an IgM positive rate of 36% versus 34%, whereas OspC from *B. garinii* performed best in neuroborreliosis (60% versus 44%). The anti-OspC IgG response was less prominent than the IgM response and was infrequent in the late stages of the disease (0–20%). The benefit of combining the evaluation of anti-OspC responses with all three species was limited. The overall diagnostic sensitivity of WB anti-*B. garinii* OspC evaluation was, in the early stages of the disease, comparable to the results obtained using the flagellum ELISA. In erythema migrans and neuroborreliosis, the addition of anti-OspC IgM to the flagellum ELISA increased the sensitivity by 15% and 10%, respectively. It can, therefore, be concluded that OspC from *B. garinii* is a suitable OspC test antigen, and that supplementary use of OspC from other species adds little to the overall diagnostic sensitivity. An ELISA based on *B. garinii* OspC and native flagella seems currently the most promising concept for a future antibody test in early Lyme borreliosis.

Key words Borrelia · OspC · Flagellum · Western blotting · Strain specific immunoreactivity

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Introduction

The tick-borne spirochete *Borrelia burgdorferi* is the etiological agent of Lyme borreliosis (LB), which is now the most common vector-borne human disease in Europe and North America [4, 21]. The clinical manifestations are diverse and may involve the skin [1], nervous system [10], heart [25], and joints [20].

Laboratory diagnosis of LB has been possible since the discovery of *B. burgdorferi* in 1982 [4]. However, the ultimate diagnostic assay has yet not been developed. Laboratory confirmation of LB still mainly relies on the detection of antibodies to *B. burgdorferi*. Assays based on whole cell *B. burgdorferi* extracts lack diagnostic specificity due to antibodies cross-reacting with antigens through a wide range of bacterial species. Standardization and general application of Western blotting (WB) has been difficult due to strain differences, the complexity of the band pattern, and inherent problems in standardization of WB in general. Efforts have, therefore, mainly been directed towards identification of single immunodominant antigens which can be purified and used as test antigens, either as native or recombinant proteins.

According to WB studies there are two main *B. burgdorferi* antigens that meet the essential criterium of eliciting an early and strong antibody response in the majority of patients. These are the *B. burgdorferi* flagellum and the outer surface protein C (OspC) [26, 28]. Purified native *B. burgdorferi* flagellum performs well in enzyme-linked immunosorbent assays (ELISAs) [11, 12] and is now the antigen of choice in many routine diagnostic laboratories.

The application and evaluation of OspC as a diagnostic test antigen is currently a topic of major interest [6–8, 16, 26, 31].

Due to substantial strain diversity, *B. burgdorferi* was recently subdivided into three genospecies called *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* [2]. Consequently strain and genospecies differences between antigens are of importance for the selection of antigens when diagnostic tests are constructed. Due to the highly conserved amino acid sequence of *B. burgdorferi* flagellin (96% identity [13, 14, 19]), the source of *B. burgdorferi* flagellum as test antigen is of minor, if any, importance. By contrast, the OspC amino acid sequence shows significant differences between the genospecies and within the species, with an amino acid sequence identity ranging from 60% to 100% (mean 75%) [13, 23, 24]. Accordingly a marked epitope diversity of OspC has been found, even using polyclonal antisera [23, 27, 29].

A high prevalence of anti-OspC IgM antibodies has been found in patients with LB using WB [7, 26, 31], and recently also by ELISA using recombinant OspC (rOspC) [7, 8, 16]. The aim of the present study was, using WB, to determine the prevalence of anti-OspC IgM and IgG antibody response to native OspC from all three genospecies in patients with different manifestations and stages of LB. No previous studies have addressed this topic systematically using such a large and well-documented series of clinical material, and *B. burgdorferi* representing the three genospecies all expressing abundant OspC. Such data are essential for the selection of the best source of OspC as an antigen for serodiagnosis. In the present study the diagnostic sensitivity and specificity of anti-OspC detection by WB was compared with the performance of the flagellum ELISA [11, 12].

Materials and methods

Borrelia strains and antibodies

Three different strains were used representing the genospecies: *B. burgdorferi* sensu stricto (MUL; kindly provided by R. C. J. Johnson, Minneapolis), *B. garinii* (DK 6), and *B. afzelii* (DK 26); DK 6 and DK 26 belonged to our own laboratory collection. All strains expressed abundant OspC. The cells were grown, harvested and stored as previously described [3]. Two OspC-specific monoclonal antibodies (mAbs) (kindly provided by Bettina Wilske, Munich) were used: L22-1F8 is broadly reactive to most strains, including the three strains used in this study; and L22-1F10, which binds OspC from most of *B. afzelii* strains, including DK 26 [29]. Mono-specific rabbit antiserum was raised against polyacrylamide gel electrophoresis (PAGE)-purified OspC from DK 6 [23]. A positive human serum from an erythema migrans (EM) patient with a strong WB reactivity to the flagellin and OspC band was used as a control in the WB set up.

Clinical material

Sera from patients with LB

We examined 117 sera from patients with clinical symptoms of untreated LB.

Sera from 47 patients with EM. The diagnosis was culture verified by skin biopsy in 22 patients, and in the remaining 25 cases the diagnosis was based on clinical evidence without previous serological testing. These sera were collected from 1989 to 1992. The median disease duration was 3 weeks and ranged from less than 1 week to one year.

Sera from 50 consecutive patients with neuroborreliosis (NB) collected in 1991. The diagnosis was based on clinical evidence; all but two patients had lymphocytic pleocytosis in CSF; in one patient CSF cytology was not examined, and in the other CSF cytology was performed after antibiotic treatment; both patients had a definite history of clinical neuroborreliosis and positive intrathecal antibody synthesis. All patients had *B. burgdorferi* specific intrathecal antibody synthesis [9]. The median disease duration was 3 weeks and ranged from 1 week to 1½ year after onset of neurological symptoms.

Sera from 20 patients with acrodermatitis chronica atrophicans (ACA). These sera were collected from 1987 to 1990. The clinical diagnosis was in every patient made by a dermatologist. The disease duration ranged from 8 months to 10 years, median 4 years.

Control sera

Sera from 29 patients with early syphilis were used. These sera all had shown to have very high IgM and/or IgG antibody levels (absorbance >1.5) in the Reiter treponeme flagellum ELISA [17, 18]. All sera were positive in WR, RPR and the FTA-absorption test. In addition, 100 randomly collected sera from danish blood donors were examined. All sera were stored at –20 °C.

Polyacrylamide gel electrophoresis and Western blotting

PAGE and WB was performed essentially as previously described [23]. Minor modifications were as follows: antigen corresponding approximately to 10^8 cells was applied to one broad slot per gel (16 cm×0.5 cm); after electroblotting the nitrocellulose (NC) membrane was cut into 4-mm strips; sera were diluted 1 : 100 and incubated with the strips at room temperature for 3 h; antibody-reactive proteins were detected with alkaline phosphatase-coupled swine anti-mouse immunoglobulins (DAKO, Denmark; cat. no. D 314), swine antirabbit immunoglobulins (DAKO cat. no. D 306), rabbit antihuman IgM (DAKO; cat. no. P 337), and rabbit antihuman IgG (DAKO; cat. no. P 336). All conjugates were diluted 1 : 1000. Identification of the OspC and p41 flagellin bands was achieved by incubating three NC strips (two lateral, one central) from every sheet with a WB-positive human control serum showing a strong reaction to both proteins. Every immunoblot reaction also included a negative human control serum. The NC membranes were incubated with substrate for 5 and 10 min for IgG and IgM, respectively.

All patient and control sera were coded and randomized, and the WB assays including the evaluation were performed blinded. The blots were only evaluated with respect to anti-OspC and anti-p41 reactivity. The reactivity was graded semiquantively: 0, no reactivity; 1, faint reactivity; 2, moderate but definite reactivity; and 3, strong reactivity. Only sera achieving a reactivity of 2 or 3 were recorded as positive.

B. burgdorferi flagellum ELISA

All sera ($n = 246$) were tested for IgM and IgG antibodies to native *B. burgdorferi* flagellum using commercial assays, a μ-capture ELISA (DAKO; cat. no. K 006), and an indirect IgG ELISA (DAKO; cat. no. K 416). Both asssays use flagella purified from strain DK 1 belonging to the genospecies of *B. afzelii.* The ELISAs were performed according to the instructions of the manufacturer, and the results were expressed as absorbance values. In both assays the diagnostic cut-off level was adjusted to a specificity of 98% based on the examination of 100 blood donors.

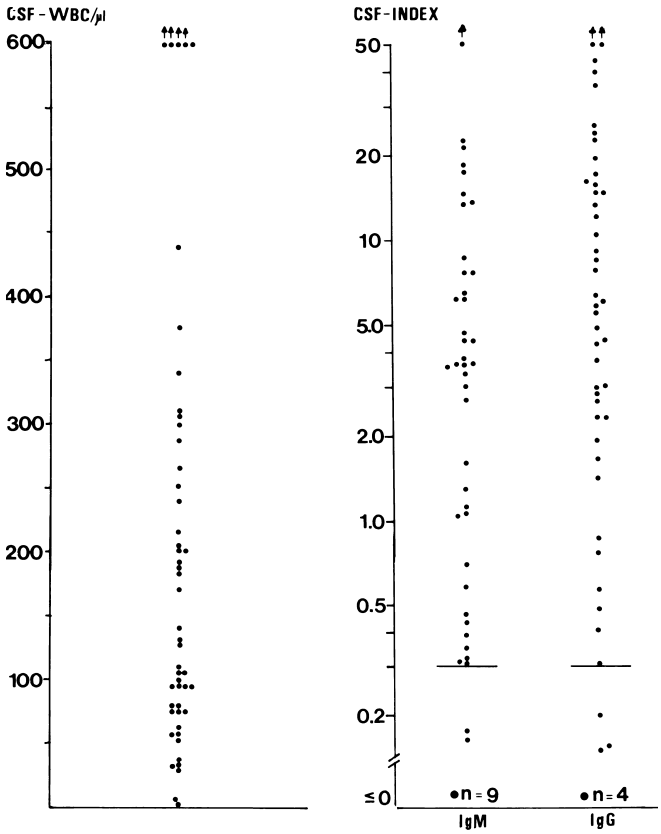

Fig. 1 Laboratory findings in the 50 neuroborreliosis patients. *Left panel* WBC counts/µl in CSF, range 5–1000 (medium 216) *Right panel Borrelia burgdorferi* flagellum-specific intrathecal IgM and IgG antibody synthesis (according to ref. 9). Index = A CSF/A serum× (A CSF-A serum); a positive index was taken at ≥0.3. (*A* absorbance, *WBC* white blood cell, *CSF* cerebrospinal fluid)

Results

Laboratory values for neuroborreliosis

The laboratory findings in the 50 consecutive neuroborreliosis (NB) patients selected for this study are summarized in Fig. 1.

Demonstration of OspC in the test strains

The authenticity of OspC in the three strains representing the three genospecies was established by WB using mAbs and polyclonal antibodies (Fig. 2). As shown in Fig. 2 all three strains expressed abundant OspC.

Frequency of antibodies to OspC variants in EM, NB, ACA, selected syphilis sera, and blood donors

The qualitative results of the blinded WB screening of the 248 human sera for antibodies against OspCs from each of the three strains are given in Table 1. It appears that *B. garinii* OspC reacted with considerably more sera than OspC from, in the order of frequency, *B. afzelii* and *B. burgdorferi* sensu stricto. This was the case in the different groups of patients except for ACA, for which *B. afzelii* OspC reacted with a higher frequency than *B. garinii* OspC. In the blood donor group, all three OspCs had a comparable low reactivity. Another observation was that the frequency of IgM reactivity in sera from patients was higher than IgG reactivity (Table 1). The selected strong Reiter treponeme flagellum-reactive syphilis sera frequently reacted with all three OspC variants, especially OspC from *B. garinii.*

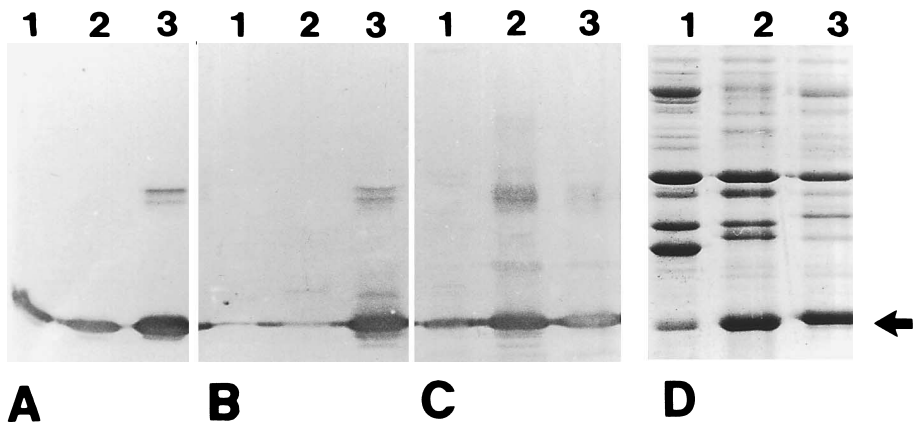

Fig. 2 Western blot (WB) demonstration of outer surface protein C (OspC) in *B. burgdorferi* sensu stricto (*lane 1*), *B. garinii* (*lane 2*), and *B. afzelii* (*lane 3*) by means of **A** a monoclonal antibody (mAb) reacting with all OspCs (mAb L22-1F8), **B** an mAb specific for *B. afzelii*-OspC (mAb L22-1F10), and **C** a rabbit antiserum raised against *B. garinii*-OspC. **D** shows the SDS-PAGE protein profiles of the three strains (*arrow:* OspC)

Table 1 Frequency (%) of WB seroreactivity of OspC variants in patients with LB and controls (*WB* Western blotting, *OspC* outer surface protein C, *LB* Lyme borreliosis)

	B. burgdorferi sensu strictu		*B. garinii*		*B. afzelii*	
	IgM	IgG	IgM	IgG	IgM	IgG
Erythema migrans ($n=47$)	19.1	4.3	36.1	25.5	34.0	10.6
Neuroborreliosis ($n=50$)	32.0	18.0	60.0	46.0	44.0	16.0
Acrodermatitis atrophicans ($n=20$)	0.0	0.0	10.0	5.0	20.0	10.0
Syphilis, selected ($n=29$)	10.3	0.0	24.1	10.3	10.3	0.0
Blood donors ($n=100$)	1.0	1.0	1.0	4.0	1.0	0.0

Table 2 Frequency (%) of erythema migrans and neuroborreliosis sera reacting with one, two or three OspC variants in Western blotting (*B.b. Borrelia burgdorferi*, sensu stricto, *B. g. B. garinii, B.a. B. afzelii*

Pattern	Reactivity against OspC from			Erythema migrans ($n=47$)		Neuroborreliosis ($n=50$)	
	B.b.	*B.g.*	*B.a.*	IgM	IgG	IgM	IgG
1	+	–	–	6.4	2.1	0.0	2.0
2	–	+	–	2.1	17.0	10.0	18.0
3	–	–	+	2.1	2.1	0.0	4.0
4	+	+	–	2.1	0.0	6.0	0.0
5	–	+	+	21.3	6.4	18.0	12.0
6	+	–	+	0.0	0.0	0.0	0.0
7	+	+	+	10.6	2.1	26.0	16.0

Frequency of EM and NB sera containing anti-OspC antibodies to one, two or all three OspC variants

All possible positivity patterns were found in individual sera, except the combination of anti-*B. burgdorferi* sensu stricto and anti-*B. afzelii* (pattern 6; Table 2). Only a few sera were non-reactive with *B. garinii* OspC and simultaneously reactive with *B. burgdorferi* sensu stricto OspC and/or *B. afzelii* OspC (patterns 1, 3 and 6; Table 2). Consequently, only a marginal increase in diagnostic sensitivity can be obtained by adding the two other OspCs to *B. garinii* OspC in an OspC diagnostic assay for EM and NB. Examples of four sera from LB patients with a genospecies-restricted antibody response by WB are shown in Fig. 3.

Frequency of sera containing antibodies to p41-flagellin in Western blotting

The results presented in Table 3 show that there was much less variation between the frequencies of sera reacting to p41-flagellin of the three genospecies than the strain-dependent variation observed in anti-OspC frequencies (Table 1). The high frequency of blood donor sera showing IgG reactivity is in accordance with previous findings in WB studies [5, 33].

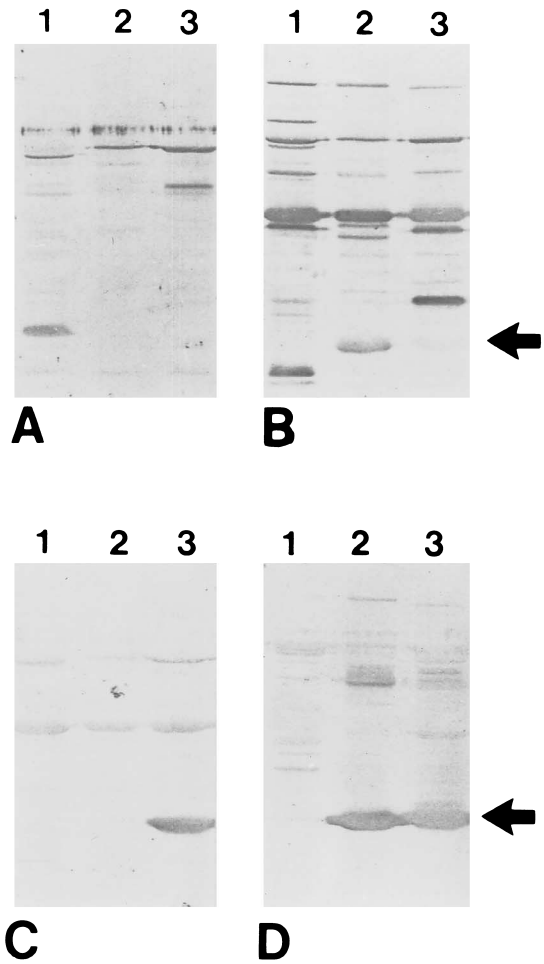

Fig. 3 A–C Three sera from patients with neuroborreliosis selected for their anti-OspC genospecies-restricted WB reactivity, and **D** one serum with reactivity against two OspCs. OspC from *B. burgdorferi* sensu stricto (*lane 1*), *B. garinii* (*lane 2*) and *B. afzelii* (*lane 3*). *Arrows* show OspC

Table 3 Frequency (%) of WB seroreactivity to the p41-flagellin band derived from the three genospecies of *B. burgdorferi*

	B. burgdorferi sensu stricto		*B. garinii*		*B. afzelii*	
	IgM	IgG	IgM	IgG	IgM	IgG
Erythema migrans ($n=47$)	38.2	57.4	27.7	61.7	34.0	42.6
Neuroborreliosis ($n=50$)	74.0	72.0	74.0	84.0	60.0	60.0
Acrodermatitis atrophicans ($n=20$)	45.0	85.0	45.0	80.0	25.0	70.0
Syphilis, selected ($n=29$)	20.7	72.4	13.8	75.9	13.8	62.1
Blood donors ($n=100$)	3.0	25.0	4.0	22.0	6.0	14.0

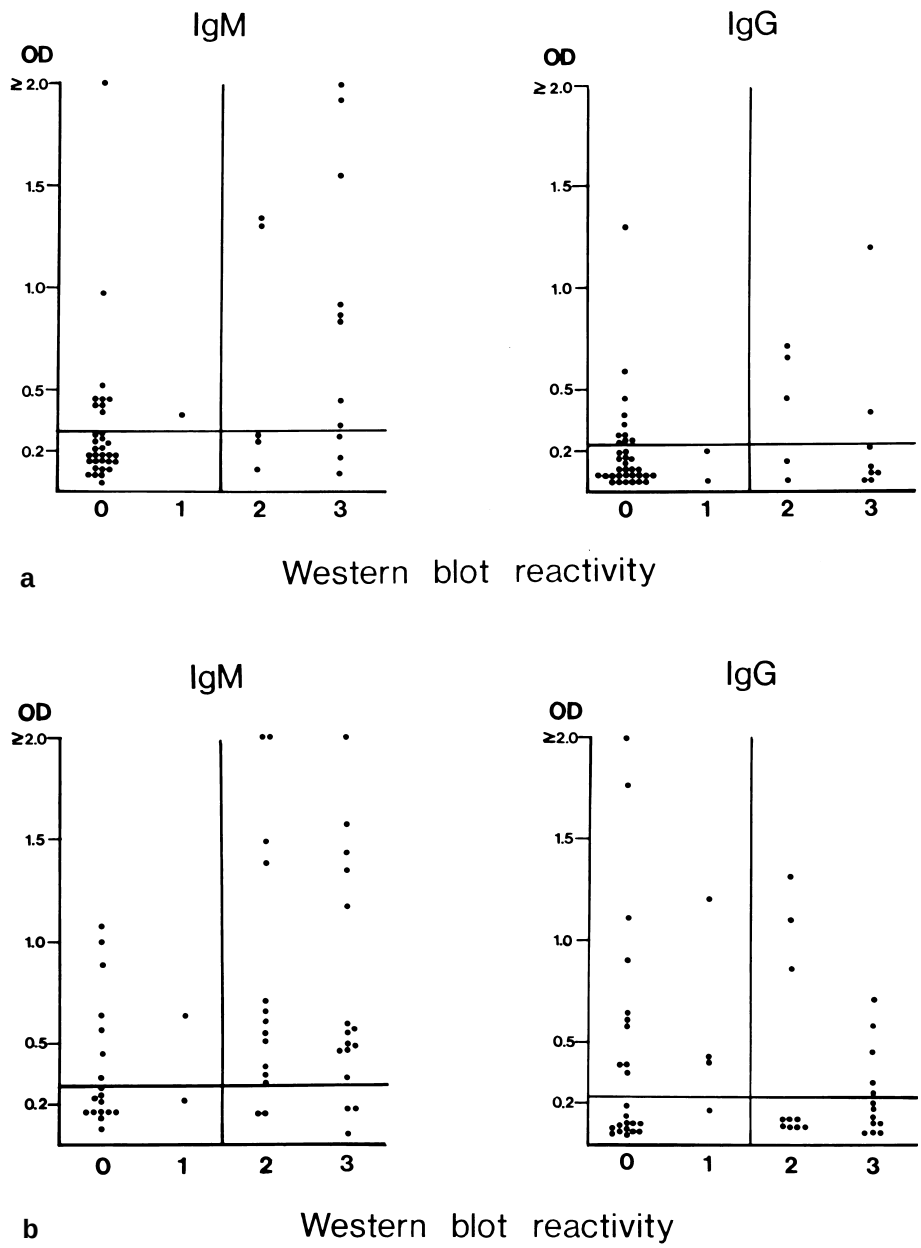

Fig. 4 Correlation between the *B. garinii* OspC WB and Flagellum ELISA in 47 patients with erythema migrans (**A**) and in 50 patients with neuroborreliosis (**B**). The *horizontal lines* mark the 98% specific diagnostic cut-off levels for the ELISAs, and the vertical lines separate the WB-negative sera (reactivities 0 and 1) from the positive sera (reactivities 2 and 3)

Table 4 Frequency (%) of seroreactivity determined by *B. burgdorferi* flagellum ELISA and *B. garinii* OspC WB

	Flagellum ELISA		*B. garinii* OspC WB	
	IgM	IgG	IgM	IgG
Erythema migrans ($n = 47$)	40.4	29.8	36.1	25.5
Neuroborreliosis ($n = 50$)	66.0	42.0	60.0	46.0
Acrodermatitis atrophicans ($n = 20$)	15.0	100.0	10.0	5.0
Syphilis, selected ($n = 29$)	0.0	89.7	24.1	10.3
Blood donors ($n = 100$)	1.0	0.0	1.0	4.0

Table 5 Comparison of the overall sensitivity of combined IgM and or IgG detection using either *B. garinii* OspC WB and/or the flagellum ELISA

Erythema migrans ($n = 47$)	Flagellum ELISA	
	IgM and IgG negative	IgM and/or IgG positive
Anti-OspC IgM and IgG negative	34.0%	27.7%
Anti-OspC IgM and/or IgG positive	8.5%	29.8%
Neuroborreliosis ($n = 50$)	**Flagellum ELISA**	
	IgM and IgG negative	IgM and/or IgG positive
Anti-OspC IgM and IgG negative	10.0%	24.0%
Anti-OspC IgM and/or IgG positive	4.0%	64.0%

Frequency of sera with flagellum antibodies as determined by ELISA

The results are summarized in Table 4, and are in accordance with results previously published [11, 12]. The very high prevalence of sera with IgG antibodies in the syphilis group may be due to the selection of sera all having a strong reaction (absorbance >1.5) in the Reiter treponeme flagellum-ELISA.

Diagnostic specificity of WB anti-OspC assays, WB anti-p41-flagellin assays, and the flagellum ELISA

According to the results obtained in for blood donors (Tables 1, 4), the WB anti-OspC assay and the flagellum ELISA achieve a comparable and high diagnostic specificity, in the range 96–100% for anti-OspCs, and 99–100% for the flagellum ELISA. The blood donor results shown in Table 3 confirm the general experience that in WB the diagnostic specificity of the anti-p41 flagellin reactivity is too low to be of any diagnostic value.

Correlation between the WB OspC and the flagellum ELISA results for EM and NB patients

A comparison of the individual results obtained for patients with EM and NB with respect to their anti-*B. garinii* OspC antibody response using WB and the quantitative measurement of IgM and IgG using the *B. burgdorferi* flagellum ELISA is shown in Fig. 4. It is evident that a significant number of patients (10–30%) revealed either a sole anti-OspC or a sole anti-flagellum antibody response. The frequencies of such exclusive antibody responses were similar for NB and EM.

By combining the WB anti-OspC evaluation and the flagellum ELISA results, the diagnostic sensitivity for IgM and IgG antibody detection can be increased by 15% and 17% in EM, and by 10% and 30% in NB, respectively (Fig. 4). Thus, the combined application of both test antigens seems to be advantageous. On the other hand, this advantage is however, reduced when (both test antigens and) a combined evaluation for IgM and IgG is applied. In that case only 8.5% of EM sera and 4% of NB sera were anti-OspC IgM or IgG positive but completely negative in the Flagellum ELISA (Table 5).

Discussion

The relevance of OspC for serodiagnosis in LB patients was first recognized and studied by Wilske et al. [26, 28]. However, in contrast to the *B. burgdorferi* flagellum which is the other important, but highly conserved, diagnostic antigen [13, 19], several studies on OspC have now disclosed a pronounced strain-dependent OspC diversity [13, 23, 24, 29], which complicates its application as a test antigen. The degree of genetic and antigenic diversity is, fur-

thermore, not limited to the three recently defined genospecies, but is high even within each genospecies, especially in *B. garinii* [24, 29]. Based on a panel of mAbs at least six different OspC serotypes were found among *B. garinii* strains [32] and, according to the deduced amino acid sequence, the degree of OspC homology within *B. garinii* ranged from 65 to 100% [24, 29]. Consequently patient sera were identified that revealed a serotype-restricted anti-OspC antibody response [23, 28], but it remained unknown how frequently this occurs. Wilske et al. [31] found that sera from 3 out of 12 patients with NB only recognized either OspC from *B. garinii* or *B. afzelii* using WB whereas no similar genotype-restricted anti-OspC response was demonstrated using WB with recombinant OspC from *B. burgdorferi* sensu stricto, *B. garinii* or *B. afzelii*. This discrepancy remains unexplained, but could be due to lack of post-translational modifications in the recombinant OspCs. Another study by Dressler et al. [6] claimed a significant and frequent genotype-restricted anti-OspC response in patients with LB. However, for the following reasons their conclusion is not convincing: (i) at least in the *B. afzelii* strain used (IP 3), no typical OspC expression was evident from the SDS-PAGE shown; (ii) none of the three strains was reported to react with an OspC-specific mAb such as L22-1F8; and (iii) they only reported on the quite rare IgG response to OspC present in only 4 out of 27 patients with NB.

Our study was designed specifically to answer questions regarding the significance of the OspC diversity for serodiagnosis. All three strains definitely expressed OspC as a major and, thus, easily identifiable protein which reacted with mAb L22-1F8. The OspC of *B. garinii* (DK 6) achieved the highest sensitivity in patients with early disease, EM and especially NB, whereas *B. afzelii* (DK 26) was more sensitive in patients with ACA. Conversely Wilske et al. [28, 31] found the OspC of *B. afzelii* (PKo) somewhat superior to the OspC from *B. garinii*, but the difference in sensitivity was less pronounced than in our study. A significant number of sera from LB patients did not recognize OspC from all three genospecies (Table 2) and thus showed a more or less genotype-restricted anti-OspC antibody response. However, only few anti-OspC antibody-positive patients did not react with OspC from *B. garinii* i. e., four out of all OspC-reactive EM sera, and all of the OspC-reactive NB sera had detectable IgM to *B. garinii* OspC (Table 2). Thus, the benefit of a combination of OspCs from all three genospecies was limited. Based on these observations we believe that OspC from *B. garinii* only may be used reliably without significant loss of diagnostic sensitivity. In terms of a potential ELISA test antigen this restriction further simplifies the issue.

The diagnostic sensitivity achieved by WB using *B. garinii* OspC in patients with EM and NB was comparable with the results obtained with the *B. burgdorferi* flagellum ELISA regarding both IgM and IgG detection (Table 4). Comparison of the WB OspC and flagellum ELISA results for IgM and IgG (Fig. 4) indicates that a significant number of patients were reactive for only one of the two assays. Thus, adding the OspC WB results to the positive rate detected using flagellum ELISA increased the overall diagnostic sensitivity, for, i. e., IgM detection in patients with EM and NB by 15% and 10%, respectively.

The diagnostic sensitivity of anti-*B. garinii* OspC IgM detection by WB was comparable to a recent WB study by Wilske et al. [30] using a recombinant OspC from *B. afzelii* and a similar cohort of patients. Regarding OspC-specific IgG, the positivity rate was considerably lower using recombinant OspC in both EM and NB. The reason for this discrepancy is unclear but Jauris-Heipke et al. [13] previously reported a weaker binding of mAbs to recombinant OspC than to native OspC.

Generally our study confirmed previous experience [26, 31] that anti-OspC antibodies are mainly found in early localized and early disseminated disease and not in late disease. Only Fung et al. [7] found a prominent IgG response to OspC in chronic US patients.

Until recently it was unclear why the prevalence of anti-OspC in patients with early Lyme borreliosis is considerably higher than would have been expected from the frequently of OspC expression in *B. burgdorferi* sensu lato grown in vitro, which is only 30 – 45% in European patient isolates and very rare in US isolates. Current observations by Schwan et al. [22] support the concept that OspC expression is temperature regulated and increases when the tick is feeding facilitating the transmission of the spirochete. Thus, a different level of expression of OspC during natural infection and in vitro may account for the apparent discrepancy. It is not known whether OspC expression ceases when the infection becomes chronic; this could explain why patients with late LB rarely have anti-OspC.

The diagnostic specificity of anti-OspC detection by WB based on blood donor sera was high and comparable to the flagellum ELISA. Considering the lack of any DNA sequence homology between *B. burgdorferi* OspC and known *Treponema pallidum* DNA sequences, the cross-reactivity with sera from patients with syphilis was unexpected, and remains unexplained. In a previous study by Wilske et al. [31] a less pronounced cross-reactivity was reported. Due to a considerable sequence homology to *B. hermsii* Vmp [15], a serological cross-reactivity would be expected, but has not so far been reported in patients with relapsing fever. This limitation, however, is from a pragmatic medical viewpoint of minor relevance, because relapsing fever is not endemic in areas with LB.

Due to the inherent difficulties of WB with respect to performance, standardization, evaluation and nonquantitative properties, an OspC ELISA would be preferable. The first attempts using recombinant OspC from *B. burgdorferi* sensu stricto have been reported [7, 8, 16]. Gerber et al. [8] found a good agreement with WB and a diagnostic sensitivity comparable to that found using a whole cell extract ELISA. The results of recombinant OspC ELISA seem promising regarding IgM detection, whereas the diagnostic sensitivity and specificity with respect to anti-OspC IgG detection were low [7].

Considering the significant amino acid sequence heterogeneity of OspCs even within one genotype, it is remark-

able that most OspC-positive patients reacted to *B. garinii* OspC. This could indicate that the most important epitopes for serodiagnosis are located in quite conserved domains. Epitope mapping of OspC using patient sera and either truncated or synthetic OspC peptides have not yet been reported.

In conclusion, this WB study has shown that in spite of the great strain-dependent antigenic variability of OspC, most of the OspC-reactive sera may be identified by using only *B. garinii* OspC as antigen. The diagnostic sensitivity of this approach in early disease was comparable to the *B. burgdorferi* flagellum ELISA. However, the two assays were complementary and in combination they increased the overall diagnostic sensitivity. Led by these results we believe that an ELISA based on OspC from *B. garinii* and native flagellum ought to be highly suitable for serodiagnosis in early LB.

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