Analysis of Antibody Response to the Outer Surface Protein Family in Lyme Borreliosis Patients

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Studies on frequencies of serum antibodies to outer surface proteins (Osps) in Lyme disease have produced conflicting results. Osps antigens (A, B, and C) enriched by butanol extraction, which aids band identification in immunoblotting, were used to test sera for IgG antibody to Osps antigens from Borrelia burgdorferi isolates from each subspecies (sensu stricto, afzelii, and garinii). Individual isolates were selected to include all five known European OspA genotypes. Of arthritis sera, 83% (n = 29), and of acrodermatitis chronica atrophicans sera, 81% (n = 26), recognized OspA, B, and/or C. Of erythema migrans sera, 23% recognized OspA and/or B alone, and a further 15% OspC alone. Only 5 (6%) of 86 sera (4 arthritis, 1 acrodermatitis chronica atrophicans, 0 erythema migrans) recognized all five OspA phenotypes tested. Marked differences in the reactions of individual sera to the various Osps were seen, which helps reveal the causes of discrepancies between previous reports.

The outer surface proteins (Osps) are the most abundant membrane proteins of Borrelia burgdorferi; they are plasmid-coded and exhibit considerable heterogeneity [1]. In the case of OspA, for example, at least six genotypes have been identified [2]; this genetic heterogeneity is a very prominent feature of European isolates. In previous studies [3–9], discrepancies in the frequencies of antibodies to Osps between groups of patients with various manifestations of Lyme disease were documented, and geographic variation has been suggested in addition [9]. It should be noted that these previous studies used a variety of B. burgdorferi isolates possessing different OspA genotypes. Obviously, further studies are required to determine the reasons for these discrepancies and to help clarify the role of the Osps molecules in the organ-specific disease processes involved.

In the present study, the IgG antibody response to Osp antigens, derived from B. burgdorferi isolates belonging to each of the subspecies sensu stricto, garinii, and afzelii, was analyzed in patients presenting with erythema migrans (EM), arthritis, and acrodermatitis chronica atrophicans (ACA).

In the case of arthritis arising subsequent to infection with the spirochete B. burgdorferi, a correlation between a specific immune response to OspA and the onset of severe and prolonged episodes of arthritis has been reported [10–13]; experiments in rodents also provided a clue that Osps may have arthritogenic potential [14]. For this reason, most attention was paid to arthritis patients and to those OspA phenotypes believed to be relevant in human disease in Europe [2], but in all cases, attention was also paid to OspB and C. For these tests, Osps extracted directly from B. burgdorferi were used; recombinant OspA and B from a single B. burgdorferi isolate (B31) were used for comparison. This study provides information of value in selecting borrelial antigens for serologic studies and helps to explain the causes of discrepancies noted between previous reports.

Materials and Methods

Borrelial isolates and culture conditions. The sources and classification of each isolate used are shown in table 1 [2, 15–17]; all were high-passage isolates (>25 subcultures). Cultures were grown in modified BSK II medium [18] at 33°C for 4–5 days until the late logarithmic phase was reached.

Antigens. Osps antigens were extracted from B. burgdorferi in aqueous form, as described previously [19]. Briefly, cultures of B. burgdorferi were harvested and disintegrated by sonication. After centrifugation, the water-insoluble pellet was resuspended in PBS and mixed with an equal volume of n-butanol (Merck, Darmstadt, Germany). After incubation and centrifugation to separate the phases, the aqueous phase, containing the Osps, was dialyzed against 5 mM 2-(N-morpholino)ethanesulfonic acid (MES buffer; Sigma, Deisenhofen, Germany), pH 6.0, and applied to a cation-exchange column (Mono-S; Pharmacia, Freiburg, Germany). The Osps, which bound to the column, could be eluted in concentrated form in a single peak. Purity was checked by SDS-PAGE (see below). Figure 1 shows the protein patterns of all antigen preparations used.

Recombinant Osps A and B (rOspA and rOspB) were derived from B. burgdorferi isolate B31 (ATCC 35210). Plasmids, bacterial hosts, growth media, and methods for the cloning and expression of rOspA have been described [20]; the expression of rOspB was...
done in essentially the same way. The products were monomeric, water-soluble proteins with molecular masses of 28 kDa (OspA, native protein 31 kDa) and 31 kDa (OspB, native protein 34 kDa), both lacking the first (N-terminal) 16 or 17 aa, respectively, of the native proteins. In addition, the full-length, lipidated versions of these molecules were also expressed and solubilized in 0.1% Triton X-100 (Sigma).

Patients and sera. Sera from 31 patients with EM, 26 patients with ACA, and 29 patients with Lyme arthritis were analyzed. The patients were from southern Germany.

The ACA patients were all diagnosed in the clinic of one of the authors (U.N.), which has considerable experience with this manifestation. Decisive criteria were a combination of the histologic findings on biopsy and a persisting, highly characteristic clinical appearance. Most of these patients were positive when tested in a B. burgdorferi polymerase chain reaction (PCR), and in some, B. burgdorferi could be cultured from the skin (all isolates obtained were Borrelia afzelii), but detection of the organism was not mandatory for diagnosis. In addition, all of these patients had positive serology by immunofluorescence or ELISA (B. burgdorferi sensu stricto isolates) and was positive in all cases. Western blotting was done with B. burgdorferi sensu stricto whole lysate antigen (virtually all sera reacted strongly with multiple bands, including p18, 25, 31, 34, 39, 41, 60–66, and 83). No selection on the basis of particular sets of bands was made. Antibiotic treatment of arthritis patients was generally started after serologic confirmation of diagnosis.

The EM patients were all examined by physicians (authors not included) experienced with this manifestation; apart from the macroscopic appearance, a history of tick bite or at least extended exposure in tick-infested areas was mandatory. We used samples collected several weeks after onset of symptoms and which had IgG antibody to 41-kDa flagellin in Western blot (B. burgdorferi sensu stricto isolate). Other reactions were not taken into account in selection. Culture or PCR detection of the organism in skin biopsies was not usually done (most patients were reluctant to cooperate). Antibiotic treatment was generally started before or at the time of serum collection.

The following control groups were each tested on five antigen preparations, as indicated in table 2. Normal controls were serum samples from 20 healthy, local residents, without any evidence of a recent infection of any type. Acute infections were serum samples from 23 patients with acute toxoplasmosis and from 17 patients with acute streptococcal infections; all samples were strongly positive in the appropriate serologic tests but negative in B. burgdorferi screening tests (immunofluorescence or ELISA on B. burgdorferi sensu stricto and B. afzelii antigens, Western blotting not done). Syphilis controls were serum samples from 17 patients with serologic evidence of a prior infection with Treponema pallidum (VDRL and fluorescent treponemal antibody absorption tests positive, IgM test negative). Results of Borrelia serology were not taken into account. Absorption with Reiter’s treponemen before use in the current study was not done.

SDS-PAGE and immunoblotting. Water-soluble Osps were separated in discontinuous SDS-PAGE (12.6% polyacrylamide gel) according to the method of Laemmli [21]. Protein concentration was measured by the method of Bradford [22]. Into each gel

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Table 1. Classification of borrelial isolates according to four different systems.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>OspA genotype</th>
<th>Serotype/genotype</th>
<th>Genotype</th>
<th>Serotype</th>
<th>Probable subspecies</th>
</tr>
</thead>
<tbody>
<tr>
<td>B31</td>
<td>Tick (United States)</td>
<td>I</td>
<td>II</td>
<td>I</td>
<td>I</td>
<td>Borrelia sensu stricto</td>
</tr>
<tr>
<td>GeHo</td>
<td>Skin (Germany)</td>
<td>I</td>
<td>II</td>
<td>?</td>
<td>?</td>
<td>B. sensu stricto</td>
</tr>
<tr>
<td>IP-1</td>
<td>CSF (France)</td>
<td>I</td>
<td>II</td>
<td>I</td>
<td>I</td>
<td>B. sensu stricto</td>
</tr>
<tr>
<td>N34</td>
<td>Tick (Germany)</td>
<td>II</td>
<td>?</td>
<td>II</td>
<td>VI</td>
<td>B. garinii</td>
</tr>
<tr>
<td>387</td>
<td>CSF (Germany)</td>
<td>II</td>
<td>I</td>
<td>?</td>
<td>VI</td>
<td>B. garinii</td>
</tr>
<tr>
<td>IP-3</td>
<td>CSF (France)</td>
<td>II</td>
<td>I</td>
<td>I</td>
<td>II</td>
<td>B. afzelii</td>
</tr>
<tr>
<td>Bo23</td>
<td>Skin (Germany)</td>
<td>IV</td>
<td>III</td>
<td>II</td>
<td>II</td>
<td>B. afzelii</td>
</tr>
<tr>
<td>Pko</td>
<td>Skin (Germany)</td>
<td>IV</td>
<td>III</td>
<td>?</td>
<td>II</td>
<td>B. afzelii</td>
</tr>
<tr>
<td>20047</td>
<td>Tick (France)</td>
<td>V</td>
<td>?</td>
<td>III</td>
<td>0</td>
<td>B. garinii</td>
</tr>
<tr>
<td>S90</td>
<td>Tick (Germany)</td>
<td>VI</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>B. garinii</td>
</tr>
</tbody>
</table>

NOTE. CSF = cerebrospinal fluid; ? = not reported.

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Figure 1. Protein patterns by SDS-PAGE of antigen preparations used for Western blotting. In some cases, OspC band has been labeled for clarity. Rec., recombinant.
Results

How frequently did arthritis, EM, and ACA sera recognize an Osp antigen? The most comprehensive studies were done in arthritis patients, in whom the presence of IgG antibody to OspA, OspB, or OspC prepared from 9 of the 10 borrelial isolates listed in table 1 was sought; EM and ACA sera were tested on a smaller, selected number of isolates, as shown in table 2. In an initial analysis, the cumulative frequencies of IgG reactivity to OspA and/or B as well as OspA and/or C were assessed (figure 2). It can be seen that ~80% of the arthritis (24/29) and ACA sera (21/26) but <40% of the EM sera (12/31) recognized one or more Osp proteins (P < .01). Isolated reactions with OspC were more frequent in cases of EM.

IgG reactivity of arthritis, EM, and ACA sera with OspA, B, and/or C from individual borrelial isolates. The frequencies of responses to OspA, OspB, or OspC separately, in each of the 3 clinical groups with the antigen preparations used, is shown in table 2. The intensity of the bands in immunoblot was on average higher with arthritis sera than with ACA sera; reactions of EM sera were generally the weakest of all (results not shown in detail).

In the case of arthritis sera, the frequency of recognition of OspA lay between 28% (387, Bo23) and 66% (IP-1) (significant differences: IP-1 vs. 387 and Bo23, P < .01; IP-1 vs. N34 and Pko, P < .05). Recognition frequencies of OspB lay between 21% (387, Pko) and 62% (IP-1), with significant differences seen between Pko and 20047 versus all others (P < .02), as well as between IP-1 versus 387 and Bo23 (P < .05). The individual Osps were sometimes recognized in isolation.

The reactions of arthritis sera to combinations of Osp antigens are shown in figure 3. OspA and/or B were most frequently recognized in B. burgdorferi sensu stricto isolates (OspA genotype I), followed by B. garinii isolates (OspA genotypes II, V, VI) and then B. afzelii isolates (OspA genotype IV) (figure 3; significant differences: OspA/B, IP-1 vs. 387, Pko, and Bo23, P < .05; OspA/B/C, IP-1 vs. 387 and Pko, P < .05). A total of 22 arthritis sera (76%) recognized OspC from at least 1 of the isolates tested. This was always accompanied by antibody to other Osps, although not necessarily those from the same isolate. With all three Osps, variation in reactivity of arthritis sera

Table 2. Frequencies of antibody responses to different OspA, B, and C antigens in patients with erythema migrans (EM), acrodermatitis chronica atrophicans (ACA), and arthritis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Osp</th>
<th>B31</th>
<th>GeHo</th>
<th>IP-1</th>
<th>N34</th>
<th>387</th>
<th>IP-3</th>
<th>Bo23</th>
<th>Pko</th>
<th>20047</th>
<th>S90</th>
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<tr>
<td>EM</td>
<td>A</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>9</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>62</td>
<td>58</td>
<td>8</td>
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<tr>
<td></td>
<td>B</td>
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<td>0</td>
<td>0</td>
<td>4</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>C</td>
<td>27</td>
<td>19</td>
<td>0</td>
<td>54</td>
<td>8</td>
<td></td>
<td></td>
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<tr>
<td>Arthritis</td>
<td>A</td>
<td>52</td>
<td>55</td>
<td>66</td>
<td>31</td>
<td>28</td>
<td>28</td>
<td>35</td>
<td>41</td>
<td>4</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>B</td>
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<td>35</td>
<td>62</td>
<td>41</td>
<td>21</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>24</td>
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<tr>
<td></td>
<td>C</td>
<td>38</td>
<td>24</td>
<td>62</td>
<td>38</td>
<td>4</td>
<td>52</td>
<td>0</td>
<td>48</td>
<td>48</td>
<td></td>
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<tr>
<td>Controls†</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection†</td>
<td>3</td>
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<td>0</td>
<td>9</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Syphilis†</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. All values are %. Controls = healthy subjects; infection = acute streptococcal or Toxoplasma infections; syphilis = Treponema pallidum infection.

* Cumulative frequencies: OspA and/or B and/or C.

For each antigen, strips were stained with dilutions of a rabbit polyclonal antibody raised to the extracted antigen, and membranes were used only when a preselected intensity of staining was obtained. Osps were identified using polyclonal rabbit anti-OspA, B, or C. Positive control sera from a small panel of patients with severe Lyme arthritis (high IgG titers by immunofluorescence and ELISA) and a normal serum sample (negative for IgG and IgM by immunofluorescence and ELISA) were also included as controls. Results were interpreted by two independent observers. A band was considered to be present when both judges designated it as clearcut. The intensities of bands corresponding to individual Osp proteins were recorded but not used for further evaluation.

Statistical analysis. This was done on data arranged into 2 × 2 contingency tables by use of Fisher’s exact test.
was seen between isolates, even within a single genotype or subspecies.

The reaction of sera from EM patients with individual Osp preparations was variable (table 2). The most frequent reactions were seen with a single isolate, S90, which has a rare OspA genotype (VI) [2]; there were no significant differences. None of the EM sera recognized Osps from a B. afzelii isolate (Pko, genotype IV, skin isolate). A total of 8 EM sera (26%) reacted with OspC; 5 of these (16%) revealed isolated reactions.

The ACA sera reacted regularly with most Osp preparations used, except for those from a B. afzelii isolate (Pko, OspA genotype IV, skin isolate; table 2). This was the only significant difference ($P < .01$). A total of 18 ACA sera (69%) recognized OspC from at least 1 isolate; as with the arthritis sera, antibody to OspC was always accompanied by antibodies to other Osps, not necessarily from the same isolate.

rOspA and rOspB from a B. burgdorferi sensu stricito isolate (B31) were available in both the truncated form (minus lipid moiety) and the full-length, lipidated versions; a comparison of the reactions of arthritis sera with these recombinant and with the butanol-extracted antigens is shown in figure 4. Differences were seen: Both lipidated and truncated rOspA were recognized by fewer than one-third of the sera reacting with butanol-extracted OspA ($P < .01$). In the case of OspB, recognition of the truncated, recombinant antigen and the butanol-extracted antigen was very similar; the lipidated antigen performed better without reaching significance (see figure 4). For comparison, a whole cell lysate of isolate B31 was also tested. The results agreed exactly with those obtained using the butanol extract.

Testing of control sera. These sera were tested on antigens from 5 different isolates, corresponding to those used with the EM and ACA sera (see table 2). Only 1 serum sample (5%) of the healthy controls reacted with Osps from a single strain (B31, B. burgdorferi sensu stricto) (table 2, figure 2). In the 40 sera from cases of acute Toxoplasma or streptococcal infections, reactions were infrequent and were directed to antigens from single strains (table 2, figure 2). The differences to all 3 Lyme patient groups were highly significant ($P < .01$). Reactions with sera from syphilis patients were slightly more frequent: 4 (24%) of 17 reacted with one or more Osp antigens. Reactions in most of the control sera were mainly borderline-positive, strong bands being the exception.

Recognition frequencies of OspA genotypes and phenotypes by arthritis, EM, and ACA sera. The frequencies with which OspA antigens of different genotype or phenotype were recognized by IgG antibody are shown in figure 5. Differences were seen: Both lipidated and truncated rOspA were recognized by fewer than one-third of the sera reacting with butanol-extracted OspA ($P < .01$). In the case of OspB, recognition of the truncated, recombinant antigen and the butanol-extracted antigen was very similar; the lipidated antigen performed better without reaching significance (see figure 4). For

Figure 2. Cumulative frequency of recognition of combinations of Osp antigens from various Borrelia isolates by erythema migrans (EM), acrodermatitis chronica atrophicans (ACA), and arthritis sera, as well as by control sera (CONT. = healthy controls; INFECT. = acute streptococcal or Toxoplasma infections; SYPHILIS = prior Treponema pallidum infection).
Recognition of combinations of Osp preparations from individual isolates by arthritis sera. (Pko) (P < .01); this was a skin isolate (figure 5). There were no significant differences in recognition of OspA of genotypes I, II, V, and VI between arthritis and ACA sera.

In contrast, EM sera failed to recognize OspA from isolates with the two most common genotypes (I and II: B. burgdorferi sensu stricto and a part of the genotypic spectrum of B. garinii) and, when positive, reacted mainly with an isolate having genotype VI (S90, B. garinii) (figure 5). The differences compared with arthritis and ACA sera were significant with all genotypes (P < .01), except between ACA and EM sera with genotype IV.

In individual sera in all 3 patient groups, reactions were usually restricted to OspA from isolates representing a limited number of genotypes. OspA from isolates covering all five European OspA genotypes were recognized by only 5 from a total of 86 sera (4 patients with arthritis, 1 with ACA; see figure 5).

Discussion

This study provides a detailed analysis of IgG antibody responses to Osps in patients with Lyme borreliosis from southern Germany, with emphasis on sera from patients with arthritis. A comparison with patients presenting with the early manifestation (EM) and the rarer, late skin lesion (ACA) was made. Sera from patients with neuroborreliosis or other clinical manifestations were not studied. Control groups included healthy subjects, patients with acute, nonborrelial infections, and syphilis patients. The isolates used were selected to cover all OspA genotypes believed to be relevant to human disease in Europe [2], and when available, within a particular genotypic group, isolates were taken from various isolation sources (tick, skin, cerebrospinal fluid).

A number of previous studies [3–10] have looked at humoral immune responses to Osps in Lyme borreliosis. For these studies, a variety of antigen preparations, including whole borrelial lysates, recombinant proteins (usually fusion proteins), and peptide fragments, were used in ELISA and Western blot systems.

In six reports [3, 5–8, 10], antigens from a single Borrelia subspecies were used. Two further studies [4, 9] used antigens from a range of B. burgdorferi isolates covering all three recognized subspecies (sensu stricto, garinii, afzelii) and diverse OspA genotypes (included genotypes I, II, IV, and V, but not all isolates were typed). A comparison of these publications reveals considerable discrepancies in the frequencies of antibodies to Osps found. In two of the above studies [6, 9], antibody to OspA was reported as being rare or absent, even in late disease, whereas in the other six reports [3–5, 7, 8, 10], antibodies to OspA and B were found frequently.
In the light of the results reported in the current study, these discrepancies can be somewhat better understood. In one report in which antibody to OspA was not found [6], OspA from isolate Pko (B. afzelii, OspA genotype IV) was used. This negative result can probably be attributed to the choice of antigen, rather than to the absence of antibody in all sera tested, as OspA from isolate Pko also behaved poorly in our hands. It was barely recognized by EM and ACA sera and belonged to the B. burgdorferi isolates whose OspA were least well recognized by arthritis sera.

In another study, Dressler et al. [9] concluded that OspA and B were rarely recognized. These patients were also German, although from different regions. Thus, geographic variation could have contributed to the discrepancies.

Perhaps these studies [6, 9] reflect, in part, the uncertainties involved in interpreting Western blots, particularly of whole borrelial lysates. This was the basic reason for using enriched Osp antigen preparations in the current study. Many approaches to improve the reproducibility of Western blotting have been tried. We controlled the efficiency of transfer of OspA, B, and C from the gel to the membrane by staining with specific antibodies. Since the relative quantities of each antigen present in a particular preparation vary, we maintained a similar concentration of OspA between preparations. The concentrations of OspB and OspC vary, therefore; however, provided a protein band was visible in SDS-PAGE, the immunoblot should be positive in the presence of antibody. We believe this approach is acceptable, provided no in-depth attempt to analyze the intensity of banding is made.

The results obtained here underline the fact that the most intensive humoral (IgG) response to OspA and OspB is to be expected in cases of arthritis, in line with a majority of other reports [3, 4, 8, 10]. In our hands, ~80% of ACA sera also recognized OspA and/or B, although these reactions were generally weaker than those seen with the arthritis sera (results not reported in detail). Cases of ACA were included in three previous studies [4, 6, 9], and in contrast to our results, antibodies to Osps were only rarely detected. The choice of antigen and the stage of the disease may have played a role. Only 23% of EM sera recognized OspA and/or B, and these reactions were much weaker. This is in line with the above reports [3, 4, 6, 7, 9, 10].

Reactions with sera from healthy controls and from patients with acute streptococcal or Toxoplasma infections were infrequent. The positive reactions almost certainly reflect the local epidemiologic situation, in which 5%-10% of the population concerned will probably have had prior contact with B. burgdorferi. Sera from patients with a history of T. pallidum infection were, as expected, more frequently positive. This result emphasizes the importance of identifying treponemal antibodies when performing Borrelia serology; none of the Lyme disease patients studied here had such antibodies. We conclude that the vast majority of the seroreactions to Osp antigens reported here are the result of a Borrelia-specific immune response.

It was also clear that marked differences in the reactivity of sera with Osps from different borrelial isolates occurred; for example, some arthritis and ACA sera showed an isolated reaction with OspC in a particular isolate but reacted with OspA or B from another isolate or isolates. Since all of our patients were from southern Germany, the results may not be generalizable to other geographic regions.

Arthritis sera showed the lowest reactivity with Osps from isolates 387 (B. garinii, OspA genotype II) and Pko (B. afzelii, OspA genotype IV). The most frequent reactions were seen with Osps from isolate IP-1 (B. burgdorferi sensu stricto, OspA genotype I). ACA sera also reacted poorly with Osps from isolate Pko (B. afzelii) but recognized Osps from other isolates with similar frequencies; in the case of EM sera, recognition of OspA and/or B was rare (<5%) in all isolates tested except isolate S90 (B. garinii, OspA genotype VI), to which 19% reacted.

It is difficult to interpret this latter result, as borrelial skin isolates in EM have not been shown to carry this genotype [17]. Perhaps OspA from isolate S90 carries more of the epitopes recognized early on than do the other OspA preparations used; such an advantage was not seen at later stages of disease. Conceivably, bias may have been introduced into the EM group by selection on the basis of serologic testing. However, if present, this would be expected to favor antigens of B. burgdorferi sensu stricto isolates. It has been shown [23] that serum antibody to OspA may be bound in immune complexes, hindering detection in early disease; this is probably a factor reducing the frequency of positive results in the current and earlier studies.
As a practical consequence to be drawn from such data, when testing for IgG antibody to OspA and OspB in cases of arthritis, a *B. burgdorferi* sensu stricto isolate can be recommended. Osp antigens from all 3 *B. burgdorferi* sensu stricto isolates tested performed better in Western blotting than did those from the other subspecies; however, the combination with *B. afzelii* and *B. garinii* isolates does have a real, if small, advantage.

In the case of ACA and EM sera, fewer isolates were tested, so that much more care must be exercised when drawing such conclusions. Between 17% and 20% of the arthritis and ACA sera and >60% of the EM sera failed to react with any of the Osp antigens used. Some of these patients will not have made antibody to Osps. In others, leaving aside the possibility of incorrect diagnosis, it is possible that samples were collected at inappropriate time points (too early or too late) or that the antigenic variability of Osp antigens is even greater than that covered here.

Individual sera from arthritis and ACA patients did not react preferentially with Osps from a particular isolate, regardless of subspecies or OspA genotype used. Usually antigens from 2 or more isolates were recognized equally well. In other words, in the vast majority of these patients, studies on serologic reactions to Osps produced no circumstantial evidence that would link a particular clinical manifestation with infection with an individual isolate or even a particular subspecies. Other investigators have also reported that it is not possible to identify the isolate responsible for infection in individual patients from analysis of immunoblots [9, 24].

On the other hand, some reports do suggest a connection between particular subspecies (selected genotypes and phenotypes) and certain clinical manifestations. For example, ACA has been associated with *B. afzelii* isolates; the association with other symptoms was less clear [4, 6, 9]. It should be noted that these latter conclusions were based either on the full pattern of serologic reactions with borrelial antigens (not restricted to Osps) or on identification of OspA genes in skin samples by PCR [25]. In this context, it is somewhat puzzling that ACA sera did not react with OspA from the *B. afzelii* isolate tested (Pko), although these ACA sera often recognized OspA from other isolates. Wilske et al. [6] also reported negative reactions with ACA sera to OspA from isolate Pko. To clarify this issue further, studies with Osps from several *B. afzelii* isolates are needed.

The third Osp studied, OspC, was recognized by ~70% of arthritis and ACA sera and 26% of EM sera. An IgG response to OspC appears to be nearly as good a marker of chronic borrelial disease as is antibody to OspA and OspB.
On the basis of studies with sera from southern German patients, we conclude that the source of antigen is important for detection of antibody to Osps in Lyme borreliosis. To reliably detect or exclude the presence of antibody to OspA, testing with antigen from several isolates is recommended.

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References