Antibody Responses to the Three Genomic Groups of *Borrelia burgdorferi* in European Lyme Borreliosis

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The antibody responses to the three genomic groups of *Borrelia burgdorferi* (*B. burgdorferi sensu stricto*, *Borrelia garinii*, and *Borrelia afzelii*) were determined in 97 German patients with various manifestations of Lyme borreliosis. The geometric mean antibody titers in each patient group, determined by ELISA, were similar with each antigen preparation. By Western blotting, however, patients with meningopolyneuritis tended to respond to more spirochetal polypeptides of *B. garinii*, the group 2 strain, whereas those with arthritis recognized more antigens of *B. afzelii*, the group 3 strain (*P < .05), as did those with acrodermatitis. Only 1 patient each with erythema migrans, arthritis, or acrodermatitis had weak reactivity with outer surface protein A (OspA), and none responded to OspB. It is concluded that differences among the three groups of *B. burgdorferi* may result in variations in the antibody response in European Lyme borreliosis.

Lyme borreliosis, which is caused by the tickborne spirochete *Borrelia burgdorferi*, is endemic in both Europe and North America [1]. The illness frequently begins with a skin lesion, erythema migrans, followed by dissemination of the spirochete to many sites [1, 2]. Weeks to months later, patients may have acute meningitis or meningopolyneuritis [3, 4], and months to years later, arthritis [5], acrodermatitis chronica atrophicans [6], or chronic neurologic abnormalities may develop [7, 8]. Although the clinical features of the disease are similar in Europe and the United States, some differences have been noted. Borrelial lymphocytoma, acrodermatitis, and encephalomyelitis have been seen primarily in Europe [8, 9], whereas widely disseminated early infection, secondary annular skin lesions, and arthritis have been found more commonly in the United States [2, 4]. In the United States, the typical early neurologic picture is Lyme meningitis with prominent headache and stiff neck [3]; in Europe, meningopolyneuritis (Bannwarth's syndrome) with severe radicular pain is more common [4]. It has been debated whether these apparent differences are due to observer variation or to actual differences in the disease.

Recent work in the classification of *B. burgdorferi* has begun to clarify the issue of geographic differences in Lyme disease [10–14]. By a variety of methods, three genomic groups of *B. burgdorferi* have now been identified [10–14].

To date, all North American strains have belonged to the first group, *B. burgdorferi sensu stricto*. Although all three groups have been found in Europe, most isolates have been group 2 and 3 strains [10–14]. According to taxonomic rules, these groups represent different genomic species. Group 2 strains have been renamed *Borrelia garinii*, and group 3 strains have been renamed *Borrelia afzelii* [11, 14, 14a]. Of the isolates tested to date, all cerebrospinal fluid isolates have been group 1 or 2 strains, whereas most skin isolates from patients with acrodermatitis have been group 3 strains [10–14].

European and US investigators have also found differences in the antibody responses of patients with Lyme borreliosis. In Europe, Wilks and colleagues [15, 16] reported that patients with erythema migrans most commonly had IgM responses to the 22-kDa outer surface protein C (OspC) and to the 41-kDa flagellar antigen of the spirochete, and most patients with meningitis had IgG bands at 22, 41, and 60 kDa [15, 16]. Ziller et al. [17] reported that bands at 21 and 41 kDa were usually the first to appear, followed by bands at 13, 18, 21, 23, 30, 39, 40, 63, 73, and 94 kDa in later stages of the infection [17]. As with European patients, we found that US patients with erythema migrans usually had IgM responses to a 21-kDa OspC and to the 41-kDa flagellar antigen [18]. The 21-kDa polypeptide in our antigen preparation reacts with monoclonal antibody L22 1F8, which is specific for OspC of the spirochete [19]. However, within months after disease onset, US patients often had reactivity with even more spirochetal polypeptides, including those at 18, 21, 28, 30, 31, 34, 39, 41, 45, 58, 66, 74, and 93 kDa [18]. It is unclear whether these differences are due to different criteria in patient selection, interlabatory variation, or antigenic differences among strains of *B. burgdorferi*.

In the current study, analogous to our recent study in US patients [18], we determined the antibody responses to the
three genomic groups of B. burgdorferi in German patients with various manifestations of Lyme borreliosis.
months after disease onset, the 27 patients with meningopolyneuritis (Bannwarth's syndrome) often had specific IgM or IgG reactivity or both. Months to years after disease onset, the 26 patients with arthritis usually had high IgG responses to the spirochete and only a few had IgM reactivity. Years after disease onset, all 15 patients with acrodermatitis had marked IgG reactivity and none had IgM responses. The geometric mean titers in each patient group were similar with each antigen preparation.

**Western blotting.** When the 97 sera were tested by Western blotting using each of the three antigen preparations, the patients with erythema migrans commonly had IgM and sometimes IgG responses to the 41-kDa flagellar antigen and to the 58-kDa heat-shock protein of the spirochete (figure 3A, B). The patients with meningopolyneuritis had IgM or IgG responses to these antigens and sometimes to those at 18, 28, 39, 74, and 93 kDa (figures 3C, D). The patients with arthritis always had IgG reactivity with the 30-, 41-, 58-, and 93-kDa proteins and sometimes with those at 18, 39, 45, and 74 kDa (figure 3E). The patients with acrodermatitis usually had reactivity with all of these antigens (figure 3F).

In patients with erythema migrans, an IgM response to the 21-kDa OspC was significantly more common with the group 1 strain and reactivity with the 41-kDa flagellar antigen was more frequent with the group 2 strain (P < 0.05; table 1). Patients with meningopolyneuritis had IgG responses more frequently to the 18-, 21-, 28-, 39-, 41-, 45-, and 74-kDa polypeptides of the group 2 strain, but the differences among the groups were not statistically significant. In contrast, patients with arthritis more often had IgG responses to the 18-, 28-, 30-, 41-, 45-, and 66-kDa antigens of the group 3 strain (P < 0.05); those with acrodermatitis also had IgG reactivity more frequently with the 28-, 30-, 41-, and 66-kDa polypeptides of that strain (P < 0.05). The differences in reactivity to the 28- and 30-kDa polypeptides were accounted for by the fact that the same sera recognized the 28-kDa polypeptide of the group 3 strain and the 30-kDa antigen of the group 1 and 2 strains. With all 3 strains, only a few sera had responses to polypeptides in the 31- to 35-kDa region, the locations of OspA and OspB. When the sera from the 97 patients were tested using recombinant constructs of these proteins, only 1 patient each with erythema migrans, arthritis, or acrodermatitis had weak reactivity with OspA and none responded to OspB.

Altogether, the mean number of bands present on IgM blots in patients with erythema migrans was only one with each of the 3 strains (table 2). Patients with meningopolyneuritis tended to have more reactivity on IgG blots with spirochetal polypeptides of the group 2 strain, whereas those with arthritis recognized more antigens of the group 3 strain (P < 0.03), as did those with acrodermatitis.

**Discussion**

In this study, strains representing the three genomic groups of *B. burgdorferi* (B. burgdorferi sensu stricto, B. garinii, and *B. afzelii*) were found to differ in the molecular masses of the known outer surface proteins, OspA, OspB, and OspC. Probable differences were also apparent in the molecular masses of polypeptides at 28 or 30 kDa and at 93 or 100 kDa. A recently described OspD protein has a molecular mass of 30 kDa [26], but we do not know whether the 28- or 30-kDa polypeptides in our antigen preparations are
OspB than those present in our recombinant proteins, but their sera almost always lacked reactivity in the 31- to 35-kDa region with antigens from sonicated whole spirochetes of all 3 strains. In contrast, in a previous study of 127 US patients with various manifestations of Lyme disease, 7% of the 80 patients with arthritis had strong IgG reactivity with OspA or OspB or both that developed near the beginning of prolonged episodes of joint involvement, from 5 months to 7 years after disease onset [25]. The combination of the HLA-DRA4 specificity and OspA and OspB reactivity was associated with chronic arthritis and lack of response to antibiotic therapy. It is not yet clear whether treatment-resistant chronic arthritis is a feature of European Lyme borreliosis.

In the current study, we did not attempt to develop criteria for seropositivity in European Lyme borreliosis, nor did we select control groups to determine the sensitivity and specificity of such criteria. However, serologic testing for B. burgdorferi would appear to be more problematic in Europe than in the United States. First, infection in Europe may occur with any one of the three genomic groups of the spirochete, and the infecting strain is usually not known [10-14]. In the current study, similar results were obtained with each strain by ELISA, but the number of bands in Western blots was variable. Second, regardless of the strain used, the antibody response in European Lyme borreliosis seems to be more restricted than in the US disease. Third, subclinical infection with B. burgdorferi appears to be more common in Europe than in the United States [21, 22]. Thus, both the sensitivity and specificity of serodiagnostic tests for Lyme borreliosis seem to be lower in Europe.

This study supports the idea that there are regional variations in Lyme borreliosis. Although skin, nervous system, or joint involvement probably occurs with infection in each of the groups of B. burgdorferi, arthritis is particularly prominent in group I strains in the United States, meningopolyneuritis may be a feature primarily of B. garinii infections in Europe, and acrodermatitis chronica atrophicans seems to be a feature of European B. afzelii infections. Thus, differences among the three groups of B. burgdorferi are probably an important reason for the regional variation in the clinical picture and immune response in this infection.

Table 1. Responses of German patients to the three genomic groups of Borrelia burgdorferi.

<table>
<thead>
<tr>
<th>Molecular mass (kDa)</th>
<th>IgM, erythema migrans (n = 29)</th>
<th>IgG, meningopolyneuritis (n = 27)</th>
<th>Arthritis (n = 26)</th>
<th>Acrrodermatitis chronica atrophicans (n = 15)</th>
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<td>26 26</td>
<td>8 80</td>
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</table>

NOTE: Data are % of patients with positive response to antigens of particular genomic group. Significant differences (P < 0.05) were noted in patients with erythema migrans to 21-kDa polypeptide, in those with arthritis to 18, 28, 36, 41, and 66-kDa antigen, and in those with acrodermatitis to 29, 30, 41, and 66-kDa proteins. *Significant vs. group I and II strains (P < 0.05).

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References

OspA. The 93-kDa antigen has been localized to the protoplasmic cylinder [27]. It is not yet clear whether these proteins vary among isolates of the same group or whether differences occur in other spirochetal proteins that appear to have the same molecular masses.

When these antigen preparations were used to determine the antibody responses to B. burgdorferi in German patients with various manifestations of Lyme borreliosis, the results obtained by ELISA were similar with each preparation. Enough antigens seem to be shared among the strains to give a similar overall response. With Western blotting, however, patients with meningopolyneuritis usually recognized more spirochetal polypeptides of B. gama, the group 2 strain, and those with arthritis or acrodermatitis reacted with more antigens of B. afzelii, the group 3 strain. In a report of 52 French patients, Assous et al. [28] found that half of those with Lyme arthritis had preferential reactivity with a group 1 isolate, half of those with Banffworth’s syndrome had more reactivity with a group 2 strain, and all patients with acrodermatitis had greater reactivity with a group 3 strain. In Germany, Wilske et al. [29] compared the responses of a patient with meningopolyneuritis, arthritis, or acrodermatitis to 5 isolates. The responses in patients with meningitis or arthritis were similar with each isolate, but the patients with acrodermatitis had more bands with the erythema migrans skin isolate PKo, a group 3 strain. Although the infecting strain is usually not known, several examples have been reported in which more bands were seen with a heterologous strain than with the homologous isolate [30]. Therefore, a larger number of bands on blots does not prove that the infection was caused by a particular strain.

Serum from the German patients in this study usually reacted with fewer polypeptides in each of the 3 strains than were recognized in the group 1 strain by US patients in a previous study [18]. However, the most prominent difference was the virtual absence of reactivity with OspA and OspB in German patients. It is possible that the sera of these patients contained antibodies to other epitopes of OspA or


