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 ($P < .03$), 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 [10–14a]. By a variety of methods, three genomic groups of *B. burgdorferi* have now been identified [10–14a]. 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 [10–14a]. 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* [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 [10–14a].

European and US investigators have also found differences in the antibody responses of patients with Lyme borreliosis. In Europe, Wilske 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]. Zöller 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, 60, 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, interlaboratory 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

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three genomic groups of *B. burgdorferi* in German patients with various manifestations of Lyme borreliosis.

Patients and Methods

Patients. Sera were obtained from 97 German patients with various manifestations of Lyme borreliosis. Most sera were originally sent for testing at the Medizinisch-Diagnostisches Laboratorium, a central reference laboratory in Cologne. These sera, which included samples from all 29 patients with erythema migrans, all 27 with meningopolyneuritis (Bannwarth's syndrome), 12 of 26 with arthritis, and 8 of 15 with acrodermatitis, came from locations throughout Germany. The remaining 21 sera from patients with arthritis or acrodermatitis came from six German physicians (see Acknowledgments). The referring physicians made clinical diagnoses of Lyme borreliosis and supplied clinical data including the date of disease onset, history of tick bite, and clinical manifestations of the infection.

Antigen preparations. Supernatants from sonicated lysates of whole spirochetes were prepared as described [20]. The group 1 strain of *B. burgdorferi*, G39/40, used in this study and in the previous study of US patients, was isolated from an *Ixodes dammini* tick in Guilford, Connecticut [21]. The group 2 strain, FRG, was isolated from *Ixodes ricinus* near Cologne [22]. The group 3 strain, IP3, was isolated from *Ixodes persulcatus* near Leningrad [23]. All 3 strains used in this study were high-passage isolates, which were classified by Richard Marconi (Rocky Mountain Laboratory, Hamilton, MT) using 16S ribosomal RNA sequence determination as described [11, 24]. The recombinant preparations of OspA and OspB used in this study were purified maltose-binding protein–Osp fusion proteins derived from group 1 strain B31 [25]. These fusion proteins contained the full-length OspA or OspB sequence without the lipid moiety or the signal sequence.

ELISA. Specific IgM and IgG antibodies to the spirochetal preparations were measured by indirect ELISA, as described [18]. Microtitration plates were coated overnight with 50 μg/mL (IgM) or 25 μg/mL (IgG) antigen preparation G39/40, FRG, or IP3. After blocking with 5% nonfat dried milk, the plates were incubated with patient sera (1:100 for IgM, 1:400 for IgG) and with alkaline phosphatase–conjugated goat anti-human IgM or IgG. The substrate, 1 mg/mL *p*-nitrophenyl phosphate with 8 μ*M* zinc chloride, was then added. The cutoff optical density readings (405 nm) were 3 SD (IgG) or 5 SD (IgM) above the mean optical density of 8 normal control samples included on the same plate. These samples were representative of 50 previously tested normal control samples. To calculate an antibody response, the value of each sample was adjusted with a standard curve made from dilutions of a known positive serum, also included on the same plate.

Western blotting. Western blotting was done using a miniblot system as described [18]. Briefly, supernatant from sonicated *B. burgdorferi*, G39/40, FRG, or IP3 (100 μg for IgM or IgM, 50 μg for IgG) or recombinant OspA or OspB (5 μg) was electrophoresed on a 10% acrylamide gel at 175 V. Gel proteins were transferred to nitrocellulose paper at 100 V for 1 h, and the nitrocellulose was cut into 2-mm strips. The strips were blocked in 5% nonfat dried milk in TRIS-buffered saline and 0.1% Tween 20 (M-TBS); they were incubated with patient sera (1:250 in M-TBS) and with alkaline phosphatase–conjugated goat antibodies to human IgM or IgG (1:3000 in M-TBS), all for 1 h. Finally, the substrate, 1 mL of *N,N*-dimethylformamide with 30 mg of nitroblue tetrazolium and 1 mL of *N,N*-dimethylformamide with 15 mg of 5-bromo-4-chloro-3-indolyl phosphate mixed in 100 mL of carbonate buffer (100 m*M* $NaHCO_3$, 1 m*M* $MgCl_2$, pH 9.8), was added for 15 min. The same positive and negative control samples were included with each assay.

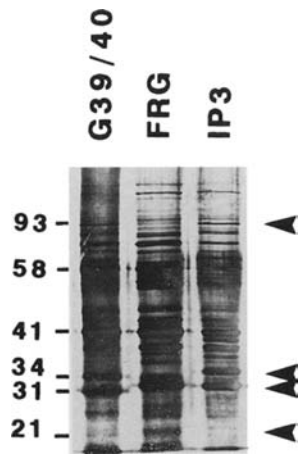

Figure 1. Colloidal gold stain of 3 *B. burgdorferi* strains: G39/40 (group 1), FRG (group 2), and IP3 (group 3). Numbers at left are molecular masses (in kDa). Arrows indicate differences among strains. Monoclonal antibodies were used to identify locations of 21-kDa outer surface protein C (OspC), the 31- and 34-kDa OspA and OspB, and 41-kDa flagellar antigen.

Statistics. The identity of groups was compared in 3 × 2 tables by χ^2 analysis, and the distribution of values among the groups was compared by *t* test. All *P* values are two-tailed.

Results

Comparison of antigen preparations. When the antigen preparations made from group 1 strain G39/40, group 2 strain FRG, or group 3 strain IP3 were stained with colloidal gold, the 41-kDa flagellar protein, the 58-, 66-, and 74-kDa heat-shock proteins, and the 37- and 45-kDa polypeptides appeared at the same locations and stained with similar intensity (figure 1). The strains differed in the migration of the known outer surface proteins. The molecular weight of OspC was 21 kDa in the group 1 strain and 22 kDa in the group 2 strain; two bands were seen in this region with the group 3 strain. OspA had a molecular weight of 31 kDa in the group 1 strain and 32 kDa in the group 2 and 3 strains. OspB migrated to 34 kDa in the group 1 strain, to 33 kDa in the group 2 strain, and to 35 kDa in the group 3 strain. The group 1 and 3 strains had a prominent 93-kDa polypeptide, whereas the group 2 strain had a pronounced 100-kDa band.

ELISA. The sera from the 97 German patients with various manifestations of Lyme borreliosis were first tested for IgM and IgG antibody responses to each of the three genomic groups of *B. burgdorferi* by indirect ELISA (figure 2). From 1 to 4 weeks after disease onset, the 29 patients with erythema migrans sometimes had specific IgM responses but usually did not have IgG reactivity. From 2 weeks to 6

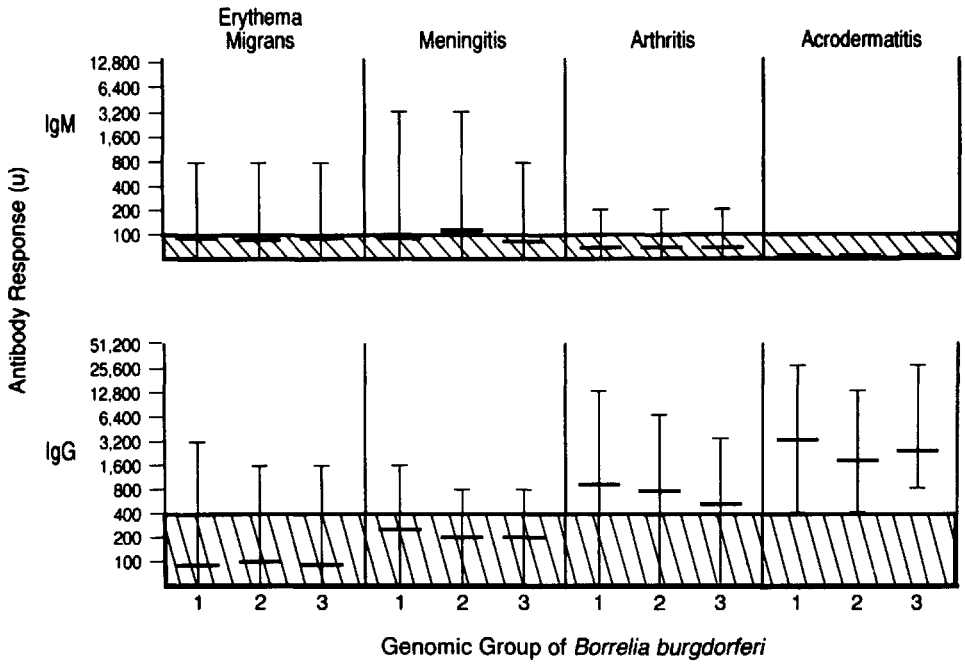

Figure 2. Antibody responses to *B. burgdorferi*, group 1 strain G39/40, group 2 strain FRG, and group 3 strain IP3, by indirect ELISA. Horizontal bars show geometric mean response; vertical bars, range; hatched areas, range of values in normal control subjects. To calculate antibody response (units), value of each sample was adjusted from standard curve made from dilutions of known positive serum.

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 almost 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 ($P < .05$; table 1). Patients with meningopolyneuritis had IgG responses more frequently to the 18-, 21-, 28-, 39-, 41-, 58-, 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 ($P < .05$); those with acrodermatitis also had IgG reactivity more frequently with the 28-, 30-, 41-, and 66-kDa polypeptides of that strain ($P < .05$). 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 ($P < .03$), 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

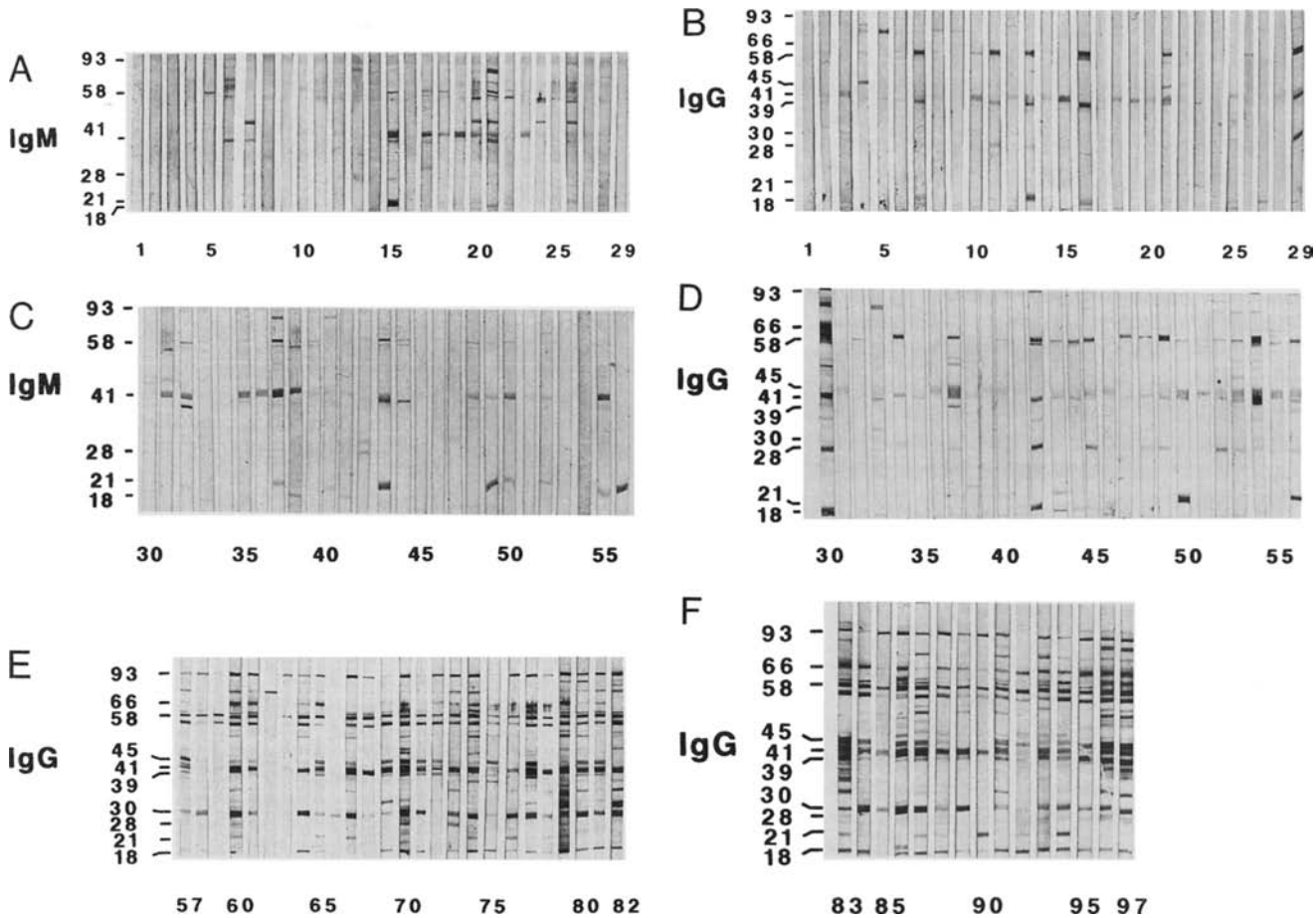

Figure 3. Western blots of patients: IgM (**A**) and IgG (**B**) blots in 29 patients with erythema migrans, IgM (**C**) and IgG (**D**) blots in 27 patients with meningopolyneuritis, IgG blots (**E**) in 26 patients with arthritis, and IgG blots (**F**) in 15 patients with acrodermatitis. For **A–D,** results with group 2 strain FRG are shown; for **E** and **F,** results are with group 3 strain IP3. Numbers at left are molecular masses (in kDa). Numbers at bottom are patient numbers.

OspD. 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. garinii*, 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 Bannwarth'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.

Sera 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

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, 71% 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-DR4 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 [31, 32]. 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 with each of the groups of *B. burgdorferi*, arthritis is particularly prominent with group 1 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*.

Molecular mass (kDa)	IgM, erythema migrans (n = 29)			IgG								
				Meningopolyneuritis (n = 27)			Arthritis (n = 26)			Acrodermatitis (n = 15)		
	1	2	3	1	2	3	1	2	3	1	2	3
18	0	7	0	4	22	15	19	58	81	73	87	87
21	41	10	0	0	15	0	8	0	27	7	0	27
28	3	0	10	4	37	33	38	15	85	27	27	87
30	7	3	3	30	7	0	73	65	23	87	73	27
31	0	0	0	0	4	0	15	4	0	0	13	0
34	3	0	3	4	4	7	19	12	35	20	7	0
37	3	7	14	26	11	4	46	19	12	40	20	20
39	3	3	7	11	22	11	31	15	38	33	53	67
41	24	62	31	67	78	44	42	73	81	53	100	93
45	24	17	17	11	7	41	8	38	54	33	53	73
58	17	17	17	52	78	56	92	88	92	93	100	100
66	3	10	21	0	7	11	4	4	38	7	7	73
74	7	10	10	11	30	19	27	31	42	20	33	40
93	7	7	7	30	26	26	85	77	81	80	80	87

NOTE. Data are % of patients with positive responses to antigens of particular genomic group. Significant differences ($P < .05$) were noted in patients with erythema migrans to 21-kDa polypeptide, in those with arthritis to 18-, 28-, 30-, 41-, 45-, and 66-kDa antigens, and in those with acrodermatitis to 28-, 30-, 41-, and 66-kDa proteins. P values were multiplied by 14 (no. of variables in this analysis).

Table 2. Frequency of bands on immunoblots in German patients with Lyme borreliosis.

Blot, patient group (n)	Genomic group		
	1	2	3
IgM, erythema migrans (29)	1 (0–8)	1 (0–8)	1 (0–6)
IgG			
Meningopolyneuritis (27)	3 (0–13)	5 (1–16)	3 (0–18)
Arthritis (26)	6 (1–18)	6 (1–17)	9 (1–18)*
Acrodermatitis (15)	9 (3–20)	10 (4–21)	12 (5–23)

NOTE. Data are mean no. of bands (range).
* Significant vs. responses to group 1 and 2 strains ($P < .03$).

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