Molecular Polymorphism of the Lyme Disease Agent *Borrelia garinii* in Northern Europe Is Influenced by a Novel Enzootic *Borrelia* Focus in the North Atlantic

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Received 2 August 1995/Returned for modification 16 October 1995/Accepted 15 November 1995

Lyme disease *Borrelia* species are distributed in temperate areas of North America and Eurasia. To elucidate the distribution of borreliae in subarctic regions, strains isolated from *Ixodes ricinus* and *Ixodes uriae* ticks found on islands in the northern Atlantic and Baltic Sea were molecularly characterized. All isolates were verified as *Borrelia garinii* by 16S rRNA gene analysis and immunoblotting with monoclonal antibodies specific for the outer surface proteins A and C. Three ribotypes (RTs) of *B. garinii* were delineated. *I. ricinus* complex-associated RT1 was phenotypically most heterogeneous. Two newly identified ribotypes were shared by different tick species and conformed to two established OspA serotypes. RT2 was restricted to the islands in the northern Baltic Sea, whereas RT3 was recovered also from ticks found in the North Atlantic. In conclusion, molecular polymorphism of the studied borrelia isolates suggests a complex enzootic potential of *B. garinii* in northern Europe and implies a novel, seabird tick *I. uriae*-associated enzootic focus of Lyme disease borreliae in the North Atlantic.

Lyme disease, the most prevalent tick-borne zoonosis in North America and Europe, is a multisystemic disorder caused by the spirochete *Borrelia burgdorferi* sensu lato (11). The infection is initiated by inoculation of the bacterium into the skin during a tick bite. Hematogenous spread of borreliae, commonly involving a variety of organs and leading to persistent dermatologic, neurologic, or arthritic complications, may occur later (25).

B. burgdorferi sensu lato is classified into three genospecies (4, 12). *B. burgdorferi* sensu stricto occurs primarily in North America and Europe, *B. afzelii* has been isolated in Europe, and *B. garinii* is encountered in Europe and in the temperate areas of Asia. *Ixodes ricinus* is the most common tick vector associated with Lyme disease in western and central Europe. The related species, *Ixodes persulcatus*, ranges from the Baltic Sea to the Pacific Ocean. The circumpolarly distributed seabird tick *Ixodes uriae* was recently found to carry the Lyme disease agent (22).

Lyme disease borreliae are genetically and antigenically polymorphic (1, 7, 31). This property is anticipated to influence regional (e.g., United States versus Europe) or *Borrelia* species-specific variation in the clinical manifestation of and immune responses during Lyme disease (3, 5, 13, 17, 26, 29, 30). Thus, the heterogeneity of borreliae has important pathogenetic, diagnostic, and preventative implications.

The major evidence for molecular polymorphism in Lyme disease *Borrelia* species comes from the molecular studies of the borrelial outer surface proteins (Osps) OspA and OspC. Recently, immunoblotting with a series of monoclonal antibodies (MAbs) against Osps provided a comprehensive serotyping system of different Lyme disease *Borrelia* isolates (28, 29).

Importantly, the identified Osp phenotypes correlated well with genospecific grouping of Lyme disease borreliae and could be confirmed by sequence analysis of the Osps (16, 27). Moreover, these studies revealed that of the three Lyme disease *Borrelia* species, *B. garinii* was phenotypically most heterogeneous.

Also, 16S rRNA sequence analysis is currently being used to define signature nucleotide positions for Lyme disease borreliae at the species level (1, 19, 20).

In this study, 16S rRNA gene analysis and Osp serotyping were utilized to characterize *Borrelia* isolates associated with different tick species on islands in the northern part of the Atlantic Ocean and the Baltic Sea. *B. garinii* was found to dominate in subarctic areas. It is suggested that molecular polymorphism of *B. garinii* in northern Europe is influenced by both a Eurasian population and a novel seabird tick-associated North Atlantic enzootic focus of this Lyme disease *Borrelia* species.

MATERIALS AND METHODS

Tick collection and isolation of borreliae. Borrelia strains included in this study were recovered from ticks collected on islands in the northern part of the Atlantic Ocean and the Bothnian Gulf of the Baltic Sea (see Table 1). People reside on Nálssöy (Faroe Islands, North Atlantic) and Norrbyskär (Bothnian Gulf), and seabird colonies are associated with four locations: Nálssöy and Flatey Island (North Atlantic) and Bonden and Malgrundet (Bothnian Gulf). No terrestrial mammals are present on Flatey Island and Bonden. Ticks were collected from the ground by flagging on Norrbyskär (9), Bonden (22), and Malgrundet or were removed from seabirds on the islands harboring seabird colonies. Ticks were identified by species and dissected for the isolation and cultivation of borreliae in BSK II medium as previously described (6, 9).

Bacterial strains. The designations and origins of borrelia strains isolated from ticks collected on islands in the northern Baltic Sea and the North Atlantic are presented in Table 1. *B. garinii* Ip90, the *I. persulcatus* tick isolate from the Asian part of Russia (18), and *B. burgdorferi* sensu lato strain Lab recovered from *I. ricinus* in Lithuania (this study) were also included in the study for comparison. *Escherichia coli* DH5 α was used for transformations with the recombinant plasmids in DNA cloning experiments.

SDS-PAGE and serotyping. Spirochetal whole-cell lysate was obtained by

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TABLE 1. Designations and origins of the studied borrelia strains

<i>Borrelia</i> isolate	Geographic origin	Biological source	No. of subcultures ^a	Reference or source
NBS23A	Norrbysskär	<i>I. ricinus</i>	4	9
NBS16	Norrbysskär	<i>I. ricinus</i>	6	9
IUB18	Bonden	<i>I. uriae</i>	8	22
IUB19 ^b	Bonden	<i>I. uriae</i>		This study
Mal01	Malgrundet	<i>I. uriae</i>	8	This study
Mal02	Malgrundet	<i>I. ricinus</i>	15	This study
Far01	Nålsöy	<i>I. uriae</i>	13	This study
Far02	Nålsöy	<i>I. uriae</i>	14	This study
Fis01	Flatey Island	<i>I. uriae</i>	12	This study

^a Number of passages prior to serotyping.

^b Only 16S rDNA analysis was performed on the total DNA extracted from the *I. uriae* tick.

boiling washed bacteria in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer. For protein profile analysis, bacterial lysates were separated by SDS-12.5% PAGE and stained with Coomassie blue R-250 (Sigma, St. Louis, Mo.). OspA and OspC were initially characterized by immunoblot with MAb H5332 (8) and rabbit polyclonal OspC-specific antiserum, respectively. Serotyping of the strains was performed by immunoblotting with an assortment of the OspA- and OspC-specific MAbs (see Tables 3 and 4) as previously described (29). In addition, OspA-specific MAbs L32 14G7 (specific for OspA of *B. afzelii*), L32 21F3, and L32 3G3 (32) were included.

16S rRNA gene (16S rDNA) analysis. A small amount of the DNA template from each *Borrelia* strain studied was obtained by boiling washed spirochetes in clean water (Millipore Water Purification System). To prevent contamination of the samples with foreign DNA, all manipulations were carried out in a single test tube in a specially allocated laboratory area and with cotton-plugged pipette tips. Similar precautions were taken to avoid carryover of amplicon in the DNA preparations during PCR experiments. The latter were always monitored by including negative control samples.

Initially, the borrelia DNA was subjected to PCR with a set of primers (the LD primer set in reference 20) targeting a *B. burgdorferi* sensu lato-specific sequence of 16S rDNA. Positive (*B. burgdorferi* B31, *B. afzelii* ACAI, and *B. garinii* Ip90) and negative (*Borrelia hermsii*) controls were included in this PCR. Subsequently, Lyme disease *Borrelia* species-specific primers (the BB, BG, and VS461 primer sets in reference 20) were utilized in another PCR to delineate species of the *Borrelia* strains studied. *B. burgdorferi* B31, *B. afzelii* ACAI, and *B. garinii* Ip90 were included as controls in this PCR. Both Lyme disease *Borrelia*-specific and Lyme disease *Borrelia* species-specific PCRs were performed as previously described (20). The amplicons generated by these two PCRs were identified by Southern hybridization with the respective internal probes (primer 16S-B and 16S-A in reference 20). The 577-bp amplicons generated by *B. garinii*-specific primers (the BG primer set in reference 20) were cloned into the T vector pUC57/T (Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. Sequence analysis of the positive clones was performed by the dideoxy chain termination method with a T7 sequencing kit (Pharmacia, Uppsala, Sweden) as described by the manufacturer. Both strands of the cloned 16S rDNA insert were sequenced by using the plasmid vector-derived primer set, the 16S rDNA primer previously described (16S-A in reference 20), and the primers designed from the sequenced DNA. The latter are the following: 5'-GCTTG TAGATGAGTCTGCG-3' (positions 216 to 234, according to the *B. burgdorferi* B31 numbering in reference 15) and 5'-CCTTGGTAGGCATTACCC-3' (positions 272 to 254 in reference 15). Variable nucleotides in the 16S rDNA sequences have been verified by sequencing cloned amplicons from a repeated PCR.

RESULTS

Isolation of spirochetes. Recovery of *Borrelia* strains from *I. ricinus* and *I. uriae* ticks collected on Norrbysskär and Bonden in the northern Baltic Sea have previously been described (9, 22). In the present study, spirochetes were cultured from sea-bird-associated *I. uriae*: 1 (6.3%) of 16 ticks from Malgrundet in the northern Baltic Sea, 2 (1.7%) of 120 ticks from Nålsöy, and 1 (1.7%) of 60 ticks from Flatey Island in the North Atlantic. Furthermore, a spirochetal isolate was obtained from 1 (4.8%) of 21 *I. ricinus* ticks collected on Malgrundet.

16S rDNA analysis. PCR amplification of 16S rDNA target sequences with a *B. burgdorferi* sensu lato-specific primer pair amplified the DNA from all borrelia strains studied, as well as *B. burgdorferi* B31, *B. afzelii* ACAI, and *B. garinii* Ip90 used as positive controls (data not shown). *B. hermsii* DNA could not be amplified by the PCR with these primers (data not shown). Genomic species determination of the isolates was performed with Lyme disease *Borrelia* species-specific primer sets. The DNA from all isolates studied, as well as *B. garinii* Ip90 used as a positive control, could only be amplified with a pair of primers specific for *B. garinii* (data not shown). The DNA from *B. burgdorferi* B31 and *B. afzelii* ACAI could not be amplified with this primer pair (data not shown).

Partial nucleotide sequences of a 16S rDNA fragment (539 bp) in between the regions targeted by the *B. garinii*-specific primer pair were obtained, and the variable and signature nucleotide positions are summarized in Table 2. The investigated borrelia strains demonstrated three patterns of variable and signature nucleotide positions in the 16S rDNA. Ribotype (RT) 1 had a single nucleotide difference compared with RT2. The representatives of RT3 showed a sequence pattern different from those of the two other sequences. Nucleotide identities of the investigated borrelia strains were compared with the sequences of *B. burgdorferi* B31 (15) and *B. afzelii* R-IP3 (20). Two other RT1 strains (Ip90 and Lab) were also included in the study for comparison. RT1 and RT2 possessed a single common signature nucleotide at position 170, whereas the RT3 signature set was defined by positions 174, 189, 252, and 451.

RT1 included isolates from related tick species which range from the Asian part of Russia (strain Ip90) to the eastern coast and the northern part of the Baltic Sea (strains Lab and NBS23A, respectively). In contrast, RT2 isolates were associated with different tick species on the neighboring islands of Norrbysskär and Bonden in the Bothnian Gulf. The geographic origin of RT3 isolates extended from Malgrundet and Bonden in the Baltic Sea to the Faroe Islands and Iceland. Notably, two RT3 strains were recovered from *I. uriae* and *I. ricinus* collected on Malgrundet. The remaining RT3 strains originated from the islands in the North Atlantic and were associated with the seabird tick *I. uriae*.

Protein composition. Coomassie blue-stained SDS-12.5%

TABLE 2. Analysis of variable and signature nucleotide positions of the partial 16S rDNA in studied borrelia strains^a

<i>Borrelia</i> isolate(s)	Nucleotide at the following 16S rDNA position ^b :									RT
	126	170	174	175	189	252	273	376	451	
Ip90, Lab, NBS23A	C	A	A	A	T	A	G	A	G	1
NBS16, IUB18	T	A	A	A	T	A	G	A	G	2
IUB19, Mal01, Mal02, Far01, Far02, Fis01	C	G	T	A	G	G	G	A	A	3
<i>B. burgdorferi</i> B31	T	G	A	G	T	A	A	G	G	
<i>B. afzelii</i> R-IP3	T	G	A	A	T	A	G	A	G	

^a Nucleotides of *B. burgdorferi* B31 and *B. afzelii* R-IP3 (15, 20) are included for comparison.

^b *B. burgdorferi* B31 numbering (15); insertion of C at position 213 has been ignored.

FIG. 1. Coomassie blue-stained borrelia whole-cell proteins separated by SDS-12.5% PAGE. The lysates used were *B. garinii* RT1 strains Ip90 (lane 1), Lab (lane 2), and NBS23A (lane 3); RT2 strains NBS16 (lane 4) and IUB18 (lane 5); and RT3 strains Mal01 (lane 6), Mal02 (lane 7), Far01 (lane 8), Far02 (lane 9), and Fis01 (lane 10). The positions of OspA and OspC are indicated by arrowheads. Molecular mass standards are shown on the left (Mw).

PAGE of borrelia whole-cell lysates is presented in Fig. 1. Expression of OspA and OspC was characterized by immunoblot analysis utilizing MAb H5332 and polyclonal OspC-specific antiserum, respectively (data not shown). All isolates expressed OspA and OspC, except for strain Far02, which lacked OspC expression. Apparent molecular weights of OspA and OspC varied among the strains of different RTs. However, within RT2 and RT3, the OspA and OspC proteins were equivalent in size, whereas within RT1, OspA and OspC were polymorphic in size (Fig. 1). Notably, none of the studied borrelia strains had a major protein migrating at a position indicative of OspB.

Osp serotyping. Reactivities of the borrelia strains with OspA-specific MABs are shown in Table 3. All strains except strain NBS23A were typed as *B. garinii* on the basis of their reactivity with the set of Lyme disease *Borrelia* species-discriminating MABs (MABs H3TS and L32 14G7) (29). All strains were nonreactive with the *B. burgdorferi* sensu stricto-specific MAB H3TS and the *B. afzelii*-specific MAB L32 14G7. Strain NBS23A was different from the other isolates, as it was not reactive with MAB L32 1C8, which usually reacts with *B. garinii*. Notably, strain NBS23A also remained nonreactive against MAB L32 1F11, which was previously shown to recognize all *B. burgdorferi* sensu lato strains so far tested (29). Further analyses of OspA of the borrelia strains were done with several other MABs (MABs H5332, L32 1G3, L32 1F7, and L32 1D11) used for discriminating between *B. garinii* strains. RT2 and RT3 strains conformed to OspA serotypes 3 and 6, respec-

tively. Two other *B. garinii* strains of RT1 diverged into OspA serotype 6 (strain Lab) and X (strain Ip90). Furthermore, the OspA serotype 6 strains of RT1 and RT3 were differentiated by using two additional MABs (L32 21F3 and L32 3G3), which reacted only with OspA of strain Lab.

The reactivities of the different borrelia strains against OspC-specific MABs are shown in Table 4. All RT1 strains demonstrated individual patterns of reactivity with MABs. Interestingly, strain NBS23A showed no reactivity against MAB L22 2B8, known to react with all 38 previously tested *B. burgdorferi* sensu lato isolates (28). Both OspA serotype 3 isolates of RT2 expressed OspC, which is in contrast to a previously observed lack of OspC expression in OspA serotype 3 strains (28). RT2 isolates reacted identically with anti-OspC MABs, whereas RT3 strains branched into two previously nondocumented OspC types (28). The first type comprised two isolates from Malgrundet in the Baltic Sea, whereas the second type included borrelia strains from Nålsoy and Flatey Island in the North Atlantic.

DISCUSSION

In this study, genotypic and phenotypic analyses were performed on borrelia strains recovered from different tick species on five islands in the northern part of the Atlantic Ocean and the Baltic Sea.

All studied strains were identified as *B. garinii* by a species-specific PCR amplification of the 16S rDNA. Remarkably, three patterns of variable and signature nucleotide positions were verified in the partial 16S rDNA sequence of the isolates. Before this study, only a single set of variable and signature nucleotides had been delineated in the *B. garinii* 16S rRNA region considered for analysis in this study (20). This previously defined RT coincides with RT1 and includes clinical and tick isolates from Germany, Switzerland, southern Sweden, and the Asian part of Russia. The RT1 strains NBS23A and Lab originated from latitudes further to the north, suggesting wide geographical distribution of this *B. garinii* RT also in the northward direction. The expansion of RT1 could be promoted by the wide vector tolerance of *B. garinii* to the two closely related tick species, *I. ricinus* and *I. persulcatus*. On the other hand, multidirectional spread of this RT may be associated with the interference of birds within the enzootic cycle of infection, either as transporters of infected ticks or as a reservoir of borreliae. For instance, several passerine bird species breeding in northern Scandinavia make their winter quarters in eastern and southeastern Asia (2).

The partial 16S rDNA sequence of RT2 of *B. garinii* was characterized by a single nucleotide difference compared with

TABLE 3. Reactivities of borrelia strains with OspA-specific MABs

Borrelia isolate(s)	RT	Reactivity with the OspA-specific MAB:										OspA serotype ^a
		L32 1F11	H3TS ^b	L32 1C8	L32 14G7 ^c	H5332	L32 1G3 ^d	L32 1F7	L32 1D11	L32 21F3	L32 3G3	
Ip90	1	+	—	+	—	—	—	—	—	—	—	X
Lab	1	+	—	+	—	+	—	—	—	+	+	6
NBS23A	1	—	—	—	—	+	—	—	+	—	—	X
NBS16, IUB18	2	+	—	+	—	+	—	+	—	—	—	3
Mal01, Mal02, Far01, Far02, Fis01	3	+	—	+	—	+	—	—	—	—	—	6

^a OspA serotype X includes strains that have MAB reactivity patterns different from those of OspA serotype 1 to 7 strains (29).

^b *B. burgdorferi* B31 was used as a positive control (29).

^c *B. afzelii* PKo was used as a positive control (29).

^d *B. garinii* PBi (OspA serotype 4) was used as a positive control (29).

TABLE 4. Reactivities of borrelia strains with OspC-specific MABs

<i>Borrelia</i> isolate(s)	RT	OspA serotype	Reactivity with the OspC-specific MAB:							OspC serotype ^a
			L22 2B8	L22 6C4	L22 22C11	L22 1F8	L22 7G5	L22 1F10	L22 6C8 ^b	
Ip90	1	X	+	+	+	—	—	—	—	X
Lab	1	6	+	+	+	—	+	—	—	12
NBS23A	1	X	—	+	+	+	+	+	—	X
NBS16, IUB18	2	3	+	+	+	+	+	—	—	8
Mal01, Mal02	3	6	+	+	+	—	+	+	—	X
Far01, Fis01	3	6	+	+	+	—	—	+	—	X

^a OspC serotype X includes strains that have MAB reactivity patterns different from those of OspC serotype 1 to 13 strains (28).

^b *B. garinii* TN was used as a positive control (28).

RT1. These two RTs shared a common signature nucleotide, which distinguished them from the RT3 isolates, as well as other Lyme disease *Borrelia* species. In contrast to the wide geographical range of RT1 borreliae, RT2 strains were found on islands in the Baltic Sea. Importantly, RT2 as well as RT3 strains were recovered from both *I. uriae* and *I. ricinus*, two tick species that occupy different ecological niches. In subarctic regions, *I. uriae* is strongly associated with the seabird colonies, present also in the northern part of the Bothnian Gulf. The Bothnian Gulf is one of the few places in the world where the distribution of *I. uriae* and *I. ricinus* overlap. This is illustrated by Malgrundet, which harbors both the seabird colonies and the pasture habitat suitable for *I. ricinus*.

The occurrence of RT3 borreliae in the Bothnian Gulf and on two islands in the North Atlantic supposes a novel, north-westward direction of the distribution of *B. garinii*. Again, migration of seabirds from the North Atlantic to the Bothnian Gulf (24) may favor the dispersal of borreliae. Thus, the *B. garinii* population in the northern part of the Baltic Sea is shaped by enzootic potentials emerging from two geographically opposite directions, the North Atlantic and Eurasia.

All RT3 strains in the northern part of the Atlantic Ocean were isolated from the seabird tick *I. uriae*. The association of *B. garinii* with this vector species in the middle of the North Atlantic region indicates a possible distribution of *B. garinii* to the North American continent. This assumption is potentiated by the fact that seabirds banded in Iceland and on the Faroe Islands are recovered along the eastern coast of North America (23). In this respect, genetic characterization of "atypical" American strains exhibiting genetic and antigenic features different from those of *B. burgdorferi* sensu stricto may offer an intriguing perspective.

B. garinii was shown to constitute 30 to 50% of *B. burgdorferi* sensu lato tick isolates from western and central Europe (21, 29). The exclusive occurrence of *B. garinii* in this study suggests a predominance of this genospecies in subarctic regions.

In this study, the borrelia ribotyping correlated well with phenotypic analysis based on reactivity against Osp-specific MABs. RT2 and RT3 isolates revealed distinct OspA serotypes. Moreover, RT2 strains were attributed to a single OspC serotype, whereas two geographically discrete groups of RT3 strains branched into two OspC types. However, unlike RT2 and RT3, RT1 isolates showed individual anti-OspA reactivities. Notably, one RT1 isolate shared OspA serotype 6 with RT3 strains, although serotype identities could be differentiated with an additional MAB. Recently, Osp serotyping was shown to be Lyme disease *Borrelia* species specific (28, 29). Results of this study validate the specificity of Osp serotyping also at the subspecies level. Furthermore, the delineation of

new ribotypes and anti-Osp reactivity patterns of *B. garinii* in this study promotes the accepted view of considerable genetic and phenotypic heterogeneity of this Lyme disease *Borrelia* species (28, 29).

Results of previous studies provide indirect evidence of epidemiologic and clinical importance of *B. garinii* isolates in the northern part of Europe. *B. garinii* strains, genotypically analogous to RT1 in this study, have been recovered from the cerebrospinal fluid of Lyme disease patients (20). In another study, RT2 strain NBS16 was strongly considered to be a cause of endemic neuroborreliosis on Norrbyskär (9). Moreover, summer residents on this island showed elevated antibody titers against strain NBS16 but not against *B. burgdorferi* sensu stricto strain B31, indicating the epidemiologic importance of *B. garinii* strains and the occurrence of a Lyme disease *Borrelia* species-specific immune response (10). It has been shown in recent studies that OspA types of borreliae invading the cerebrospinal fluid correspond to the heterogeneity of OspA in the geographically related tick strains (14, 29). Thus, the diversity of Osps observed among the borrelia strains from small geographic areas included in this study has important implications for the diagnosis of Lyme disease. In particular, high polymorphism of OspC should be considered, since this protein is an immunodominant antigen of the early immune response (31). Moreover, since the strains of OspA serotypes 3 and 6 have been isolated from *I. uriae* (this study) and from human cerebrospinal fluid (29) as well, it appears likely that *I. uriae* is a potential carrier of human pathogenic borrelia strains. However, the epidemiologic and clinical importance of *I. uriae*-associated *B. garinii* in the North Atlantic populations awaits further investigation.

ACKNOWLEDGMENTS

We thank A. G. Barbour for MABs H5332 and H3TS and T. G. Schwan for anti-OspC polyclonal antibody. We are indebted to Virginius Tunaitis for technical assistance and to Debra Milton for carefully reading the manuscript.

This work was supported by the Swedish Medical Research Council (07922), the Swedish Council for Forestry and Agricultural Research (23.0161), the Swedish Research Council for Engineering Sciences (92-965), and the Swedish Society for Medical Research.

REFERENCES

- Adam, T., G. S. Gassmann, C. Rasiyah, and U. B. Göbel. 1991. Phenotypic and genotypic analysis of *Borrelia burgdorferi* isolates from various sources. *Infect. Immun.* **59**:2579–2585.
- Alerstam, T. 1982. Bird migration. Signum, Lund, Sweden.
- Assouss, M. V., D. Postic, G. Paul, P. Nénot, and G. Baranton. 1993. Western blot analysis of sera from Lyme borreliosis patients according to the genomic species of the *Borrelia* strains used as antigens. *Eur. J. Clin. Microbiol. Infect. Dis.* **12**:261–268.

4. Baranton, G., D. Postic, I. Saint Girons, P. Boerlin, J.-C. Piffaretti, M. Assous, and P. A. D. Grimont. 1992. Delineation of *Borrelia burgdorferi* sensu stricto, *Borrelia garinii* sp. nov., and group VS461 associated with Lyme borreliosis. *Int. J. Syst. Bacteriol.* **42**:378–383.
5. Barbour, A. G. 1984. Immunochemical analysis of Lyme disease spirochetes. *Yale J. Biol. Med.* **57**:581–586.
6. Barbour, A. G. 1984. Isolation and cultivation of Lyme disease spirochete. *Yale J. Biol. Med.* **57**:521–525.
7. Barbour, A. G., R. A. Heiland, and T. R. Howe. 1985. Heterogeneity of major proteins in Lyme disease borreliae: a molecular analysis of North American and European isolates. *J. Infect. Dis.* **152**:478–484.
8. Barbour, A. G., S. L. Tessier, and W. J. Todd. 1983. Lyme disease spirochetes and ixodid tick spirochetes share a common surface antigenic determinant defined by a monoclonal antibody. *Infect. Immun.* **41**:795–804.
9. Bergström, S., B. Olsén, N. Burman, L. Gothefors, T. G. T. Jaenson, M. Jonsson, and H. A. Mejlon. 1992. Molecular characterization of *Borrelia burgdorferi* isolated from *Ixodes ricinus* in northern Sweden. *Scand. J. Infect. Dis.* **24**:181–188.
10. Bunikis, J., B. Olsén, G. Westman, and S. Bergström. 1995. Variable serum immunoglobulin responses against different *Borrelia burgdorferi* sensu lato species in a population at risk for and patients with Lyme disease. *J. Clin. Microbiol.* **33**:1473–1478.
11. Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis. 1983. Lyme disease—a tick borne spirochetosis? *Science* **216**:1317–1319.
12. Canica, M. M., F. Nato, L. du Merle, J. C. Mazie, G. Baranton, and D. Postic. 1993. Monoclonal antibodies for identification of *Borrelia afzelii* sp. nov. associated with late cutaneous manifestations of Lyme borreliosis. *Scand. J. Infect. Dis.* **25**:441–448.
13. Dressler, F., R. Ackermann, and A. C. Steere. 1994. Antibody responses to the three genomic groups of *Borrelia burgdorferi* in European Lyme borreliosis. *J. Infect. Dis.* **169**:313–318.
14. Eifert, H., A. Ohlenbusch, H.-J. Christen, R. Thomssen, A. Spielman, and F.-R. Matuschka. 1995. Nondifferentiation between Lyme disease spirochetes from vector ticks and human cerebrospinal fluid. *J. Infect. Dis.* **171**:476–479.
15. Gazumyan, A., J. J. Schwartz, D. Liveris, and I. Schwartz. 1994. Sequence analysis of the ribosomal RNA operon of the Lyme disease spirochete, *Borrelia burgdorferi*. *Gene* **146**:57–65.
16. Jauris-Heipke, S., G. Liegl, V. Preac-Mursic, D. Rössler, E. Schwab, E. Soutschek, G. Will, and B. Wilske. 1995. Molecular analysis of genes encoding outer surface protein C (OspC) of *Borrelia burgdorferi* sensu lato: relationship to *ospA* genotype and evidence of lateral gene exchange of *ospC*. *J. Clin. Microbiol.* **33**:1860–1866.
17. Karlsson, M. 1991. Antibody response against autologous and heterologous isolates of *Borrelia burgdorferi* in four patients with Lyme neuroborreliosis. *Eur. J. Clin. Microbiol. Infect. Dis.* **10**:742–745.
18. Kryuchanov, V. N., E. I. Korenberg, S. V. Scherbakov, Y. V. Kovalevsky, and M. L. Levin. 1988. Identification of *Borrelia* isolated in the USSR from *Ixodes persulcatus schulze* ticks. *J. Microbiol. Epidemiol. Immunobiol.* **12**:41–44.
19. Marconi, R. T., and C. F. Garon. 1992. Identification of a third genomic group of *Borrelia burgdorferi* through signature nucleotide analysis and 16S rRNA sequence determination. *J. Gen. Microbiol.* **138**:533–536.
20. Marconi, R. T., and C. F. Garon. 1992. Development of polymerase chain reaction primer sets for diagnosis of Lyme disease and for species-specific identification of Lyme disease isolates by 16S rRNA signature nucleotide analysis. *J. Clin. Microbiol.* **30**:2830–2834.
21. Nohlmans, L. M. K. E., R. de Boer, A. E. J. M. van den Bogaard, and C. P. A. van Boven. 1995. Genotypic and phenotypic analysis of *Borrelia burgdorferi* isolates from The Netherlands. *J. Clin. Microbiol.* **33**:119–125.
22. Olsén, B., T. G. T. Jaenson, L. Noppa, J. Bunikis, and S. Bergström. 1993. A Lyme borreliosis cycle in seabirds and *Ixodes uriae* ticks. *Nature (London)* **362**:340–342.
23. Petersen, A. 1982. Icelandic seabirds, p. 15–60. In A. Gardarson (ed.), *Icelandic birds*. Rit Landverndar 8, Reykjavik, Iceland.
24. Staav, R. 1995. Sillgrissla i Bottenhavet. *Fauna Flora* **2**:25. (In Swedish.)
25. Steere, A. C. 1989. Lyme disease. *N. Engl. J. Med.* **321**:586–596.
26. van Dam, A. P., H. Kuiper, K. Vos, A. Widjojokusumo, B. M. de Jongh, L. Spanjaard, A. S. P. Ramselaar, M. D. Kramer, and J. Dankert. 1993. Different genospecies of *Borrelia burgdorferi* are associated with clinical manifestations of Lyme borreliosis. *Clin. Infect. Dis.* **17**:708–717.
27. Will, G., S. Jauris-Heipke, E. Schwab, U. Busch, D. Rössler, E. Soutschek, B. Wilske, and V. Preac-Mursic. 1995. Sequence analysis of *ospA* genes shows homogeneity within *Borrelia burgdorferi* sensu stricto and *Borrelia afzelii* but reveals major subgroups within the *Borrelia garinii* species. *Med. Microbiol. Immunol.* **184**: 73–80.
28. Wilske, B., S. Jauris-Heipke, R. Lobentanz, I. Pradel, V. Preac-Mursic, D. Rössler, E. Soutschek, and R. C. Johnson. 1995. Phenotypic analysis of outer surface protein C (OspC) of *Borrelia burgdorferi* sensu lato by monoclonal antibodies: relationship to genospecies and OspA serotype. *J. Clin. Microbiol.* **33**:103–109.
29. Wilske, B., V. Preac-Mursic, U. B. Göbel, B. Graf, S. Jauris, E. Soutschek, E. Schwab, and G. Zumstein. 1993. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal antibodies and OspA sequence analysis. *J. Clin. Microbiol.* **31**:340–350.
30. Wilske, B., V. Preac-Mursic, G. Schierz, W. Gueye, P. Herzer, and K. Weber. 1988. Immunochemische Analyse der Immunantwort bei Spätmanifestationen der Lyme Borreliose. *Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A* **267**:549–558.
31. Wilske, B., V. Preac-Mursic, G. Schierz, and K. von Busch. 1986. Immunochemical and immunological analysis of European *Borrelia burgdorferi* strains. *Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A* **263**:92–102.
32. Wilske, B. Unpublished data.