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Expression and sequence diversity of the complement regulating outer surface protein E in *Borrelia afzelii* vs. *Borrelia garinii* in patients with erythema migrans or neuroborreliosis

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ABSTRACT

Outer surface protein E (OspE) is a complement factor H-binding virulence factor of borrelial subspecies. It is usually absent from *in vitro* grown *Borrelia garinii*, although *in vivo B. garinii* causes neuroborreliosis (NB). We analyzed the presence and sequence spectrum of the *ospE* genes *in vivo* in *Borrelia* spirochetes. DNA samples from the skin, serum and cerebrospinal fluid (CSF) of patients with infections caused by *Borrelia afzelii* or *B. garinii* were studied, and anti-OspE antibodies in the corresponding patient sera were detected by IgG ELISA using recombinant OspE as an antigen. *ospE* genes were found in 20 of 23 erythema migrans (EM) skin biopsies with *B. afzelii*, in 2 EM skin biopsies with unknown underlying subspecies, in 5 of 9 EM biopsies with *B. garinii*, and in 1 of 4 CSF samples of NB patients with *B. garinii* infection. All OspE sequences from *B. garinii* samples were identical. In contrast, OspE of *B. afzelii* origin showed more variation. Anti-OspE antibodies were found in 8/21 (38.0%) sera from patients with *B. afzelii*-associated EM. In conclusion, our results indicate that all borrelial subspecies, but not necessarily all strains, causing human infections can carry *ospE* genes to protect themselves against complement attack *in vivo*.

1. Introduction

Lyme borreliosis (LB) is caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato group. The main causative subspecies are *Borrelia afzelii*, *Borrelia garinii* and *B. burgdorferi* sensu stricto, although new subspecies *Borrelia valaisiana*, *Borrelia spielmanii* [1], *Borrelia lusitaniae* and *Borrelia bissettii* [2] have also been reported in Europe. A typical feature of *Borrelia* spirochetes is their ability to escape immune clearance and survive for a long time in their animal hosts. To protect themselves and to avoid attack by the complement (C) system, *Borrelia* spirochetes bind C regulatory proteins to their surfaces. Serum-resistant *B. burgdorferi* sensu stricto and *B. afzelii* have been shown to be able to bind the C inhibitors factor H (FH) and factor H-like protein 1 (FHL-1), which are important fluid-phase regulators of the alternative C pathway [3]. The binding of FH to the borrelial surface occurs via two main proteins, outer surface protein E (OspE) and BbA68, also called C regulator-acquiring surface protein-1 (CRASP-1) [4–6] or more recently CspA [7].

Several plasmid-encoded OspE paralogs have been shown to be responsible for FH binding to *Borrelia* and to mediate C resistance [8,9]. We have shown that both the N-terminus and the C-terminus of OspE can interact with the C-terminal FH short consensus repeats (SCRs) 19–20 [10]. CspA is a 27.5 kDa protein that has been found to be important for borrelial survival in serum [6,11]. CspZ or CRASP-2, another proposed FH binding protein, does not seem to play a role in serum resistance [12]. Additional FH-binding proteins, variably called as ErpA (CRASP-5), ErpC (CRASP-4) and ErpP (CRASP-3) all belong to the OspE-family and are produced during *B. burgdorferi* sensu stricto infection [13,14].

Like many bacterial virulence factors, the 19.2 kDa lipoprotein OspE is plasmid-encoded. The gene is located in the circular plasmid (cp32) in a single operon with the outer surface protein F (OspF) gene. A single bacterium can, however, have multiple cp32 plasmids and thereby multiple *ospE* genes. The *ospE* gene, located at the 5′ end of the operon, is 513 nucleotides in length and encodes a 171-amino-acid protein [15]. The expression of OspE is up-

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regulated, when the temperature shifts from 23 °C in the tick to 35–37 °C in the mammalian host [16]. This feature is compatible with the need of the spirochete to resist C attack in the warm-blooded host.

Unlike the other borrelial subspecies, *B. garinii* isolates have usually not been found to express FH-binding OspE proteins [8,17], when studied *in vitro*. However, *ospE* genes have recently been found and cloned from *B. garinii* isolates [18]. IgG class anti-OspE antibodies have also been shown in the sera and cerebrospinal fluids (CSF) of patients with LB of *B. garinii* origin [19]. The two latter facts suggest that *B. garinii* subspecies causing neuroborreliosis (NB) could express OspE *in vivo*. This could explain, at least in part, how *B. garinii* can invade central nervous system (CNS) and cause a chronic infection in the form of NB. Of the *B. garinii* OspA serotypes, strains of serotype 4 have been shown to be serum resistant, while the serotypes 3 and 5–7 were serum sensitive [20]. Serotype 4 has been shown to be the most common *B. garinii* serotype causing CNS infection and having tropism for the CNS [21]. Interestingly, *B. garinii* OspE serotypes 3 and 5–7 are, however, also able to infect the CNS suggesting that these strains could also utilize complement evasion mechanisms *in vivo*. Overall, however, the reasons and mechanisms whereby *Borrelia* spirochetes can escape immune attack, invade the CNS and cause a chronic infection remain an important focus for research.

The aim of this study was to analyze the role of the borrelial C evasion molecule OspE in natural human infections. Only a limited number of OspE sequences are available from *B. garinii* and *B. afzelii*, the most common causative agents of LB in Europe. Thus, we wanted to analyze the sequence spectrum of OspE in clinical samples. Secondly, besides simultaneous *Borrelia* species identification by PCR for outer surface protein A (OspA), we wanted to amplify borrelial *ospE* gene from human skin biopsies and CSF samples. The presence of *ospE* DNA in patient samples with *B. garinii* infection would suggest that *B. garinii* also has the means to avoid C attack *in vivo* by binding FH via OspE proteins.

2. Results

2.1. OspA subtyping

Our first aim was to determine the causative *Borrelia* species from patients with erythema migrans (EM). Because of the heterogeneity of the OspA of *B. burgdorferi* sensu lato, the *Borrelia* species of the skin biopsies could be determined by PCR for OspA. *ospA* sequences were analyzed from DNA samples of the 23 fresh EM skin biopsies by using specific primers. In 2 of the 23 DNA samples *ospA* sequence and the underlying subspecies could not be determined. The 21 *ospA* sequences were all of *B. afzelii* origin and surprisingly homogeneous with only one nucleotide difference in one of the 21 sequences. This nucleotide difference was located at position 350 (from the beginning of the full-length nucleotide sequence) with a switch from A to G. Otherwise, the sequences were identical with OspA of *B. afzelii* strain PKo (GenBank accession number X65599) except for switches from G to A at position 222 and from G to A at position 283.

2.2. OspE sequences

OspE is a borrelial immune evasion molecule that binds complement FH. Partial *ospE* sequences (OspE13-108) were analyzed from the DNA of human skin biopsies and CSF samples, and additionally, for comparison, from the sera of 2 puffins. These samples included DNA from both *B. afzelii* and *B. garinii* subspecies. The DNAs of the all originally selected skin biopsies were from *B. afzelii* subspecies. To examine samples with *B. garinii* subspecies and to examine also other than skin samples, DNA of the CSF of NB patients [22] and of the sera of puffins were studied [23].

ospE DNA was found in 18 out of 21 EM biopsies of *B. afzelii* origin and in the 2 EM biopsies with undetermined subspecies (from Helsinki), in 5 out of 9 EM samples of *B. garinii* origin, in both of the 2 other EM samples of *B. afzelii* origin, in 1 (LU59) of the 4 *B. garinii* DNA samples isolated from the CSF of NB patients but in none of the puffin samples (Table 1). *ospE* DNA was not found in any of those skin biopsies from suspected EM that were originally negative with the diagnostic OspA-based PCR. Interestingly, all of the 6 *ospE* sequences originating from the *B. garinii* subspecies were totally identical while *ospE* genes originating from *B. afzelii* had more sequence variation (Fig. 1). The overall identity of *ospE* among the *B. afzelii* strains varied between 68.6% and 100%. In total, 8 different sequences of OspE in *B. afzelii* were detected. The identity of *ospE* between *B. afzelii* and *B. garinii* strains varied from 62.3% to 79.1%. *B. afzelii* strain PGau (AF029910) showed 72.4%–97.7% identity with our *B. afzelii ospE* sequences. *ospE* sequences of our *B. garinii* strains had 82.8% identity with *B. garinii* strain IP90 (AF029912). In comparison, *ospE* sequences from *B. burgdorferi* sensu stricto strains B31 (L78248), 297 (AF023852) and N40 (L13924) showed 76.4–87.0% identity with each other. *ospE* of *B. burgdorferi* sensu stricto N40 showed 47.7–67.4% identity with our *B. afzelii ospE* sequences and 60.3% identity with our *B. garinii ospE* sequences.

2.3. Serum anti-OspE antibodies

To detect anti-OspE antibodies in LB patients an IgG ELISA was set up and the recombinant full-size OspE protein originating from *B. burgdorferi* sensu stricto strain N40 was used as an antigen. This antigen has in earlier studies detected anti-OspE antibodies in infections caused by all 3 genospecies of *B. burgdorferi* sensu lato [19]. Optical density (OD) values above the mean plus 3 SD values of healthy blood donors were defined as indicative of the presence of anti-OspE antibodies. Sera were available from the 21 EM patients with proven *B. afzelii* infection and from the 2 EM patients with undetermined disease causing subspecies. Of these 23 samples, 10 (43.5%) were positive for anti-OspE antibodies (both of the 2 samples with undetermined subspecies were positive for anti-OspE antibodies). Two out of 11 (18.2%) originally OspA-PCR-negative samples had also antibodies to OspE. None of the healthy blood donors had anti-OspE antibodies (Fig. 2).

2.4. Correlation of anti-OspE and anti-flagella antibodies

To evaluate the potential diagnostic value of anti-OspE antibodies we correlated their levels with those of anti-flagella antibodies, commonly used in diagnostics. The levels of anti-flagella IgG antibodies were available from the 21 EM patients with a disease of *B. afzelii* origin and from the 2 EM patients with unknown underlying subspecies. Antibodies against OspE and flagella were analyzed from the very same serum samples. The correlation coefficient (r) between levels of anti-OspE and anti-flagella antibodies was 0.829 (Fig. 3). Four of the 23 patients had elevated levels of anti-flagella

Table 1
PCR analysis of the various patient groups for the presence of *ospE* DNA.

	EM_{afz}-skin[a] ($n = 23$)	EM_{gar}-skin[b] ($n = 9$)	NB_{gar}-CSF[c] ($n = 4$)	Puffin-serum ($n = 2$)
ospE gene found	20	5	1	0

[a] EM_{afz}-skin = erythema migrans with *B. afzelii* infection (skin biopsy).
[b] EM_{gar}-skin = erythema migrans with *B. garinii* infection (skin biopsy).
[c] NB_{gar}-CSF = neuroborreliosis with *B. garinii* infection (cerebrospinal fluid).