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Complementation of a *Borrelia afzelii* OspC mutant highlights the crucial role of OspC for dissemination of *Borrelia afzelii* in *Ixodes ricinus*

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Abstract

Alteration of the outer surface protein (Osp) composition – especially that of OspA and OspC – seems to be important for the adaptation of *Borrelia burgdorferi* sensu lato to its endothermic hosts (mammals) and poikilothermic vectors (ticks). OspA possibly mediates adherence to tick midgut cells thus enabling the borreliae to survive in the vector, while OspC is associated with borrelial invasion of the tick salivary glands and infection of the mammalian hosts. Here we describe the first successful transformation and complementation of a *Borrelia afzelii ospC* mutant with the wild-type *ospC* in trans. To test the influence of OspC on the dissemination behavior in ticks, unfed *Ixodes ricinus* nymphs were artificially infected by capillary feeding either with *B. afzelii* wild type, the *B. afzelii ospC* mutant or the *ospC*-complemented clone. Tick midguts and salivary glands were investigated after different time intervals for the presence of borreliae and for OspA and OspC by immunfluorescence staining with monoclonal antibodies. While the *B. afzelii* wild-type strain exhibiting abundant OspC on its surface disseminated to the salivary glands, the OspC-negative mutant was only present in the tick midguts. The *ospC*-complemented clone which constitutively expresses the wild-type *ospC* was again able to colonize the salivary glands. This finding demonstrates that OspC is crucial for dissemination of *B. afzelii* from the tick midgut to the salivary glands, a prerequisite for infection of the warm-blooded host. A summary of the detailed data presented here has already been given in Goettner et al. [2006. OspC of *B. afzelii* is crucial for dissemination in the vector as shown by transformation and complementation of a European OspC-deficient *B. afzelii* strain. Int. J. Med. Microbiol. 296S1(Suppl. 40), 122–124].

Keywords: *Borrelia afzelii*; Transformation; OspC deficiency; Complementation; Tick

Introduction

Lyme borreliosis caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex is the most common vector-borne disease in the United States and Europe. The *B. burgdorferi* s.l. complex comprises at least three human-pathogenic species in Europe, *B. burgdorferi*

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sensu stricto (s.s.), the only species causing Lyme disease in the United States, *Borrelia afzelii*, and *Borrelia garinii* (Baranton et al., 1992; Burgdorfer et al., 1982; Wang et al., 1999). These spirochetes are maintained in their natural setting through complex enzootic cycles between warm-blooded hosts and ixodid tick vectors (Benach et al., 1987; de Silva and Fikrig, 1995; Gern et al., 1990; Leuba-Garcia et al., 1998; Ribeiro et al., 1987; Schwan and Piesman, 2000). During tick feeding, *B. burgdorferi* migrates through the gut wall, disseminates and invades various tissues, including the salivary glands wherefrom they are transmitted to the host by saliva. During borrelial dissemination from vector to host, the spirochetes have been shown to differentially regulate several lipoproteins possibly in response to the rapid environmental changes (Fingerle et al., 1998, 2002; Leuba-Garcia et al., 1998; Schwan and Piesman, 2000). In particular the outer surface protein (Osp) C is upregulated with rising temperature and decrease of pH (Carroll et al., 1999; Schwan and Piesman, 2000) and seems to be a crucial factor in the initial events of the infection process. However, the precise function of this protein in *B. burgdorferi* still remains to be elucidated. Genetic analyses of bacterial pathogens are an important tool for identification and characterization of factors involved in pathogenesis. To ascertain the role of certain gene products for disease, the Koch's molecular postulates have to be applied (Falkow, 1988). In *B. burgdorferi* s.s., the recent identification of erythromycin and kanamycin resistance as useful genetic markers, the isolation of null mutants, and the development of extrachromosomal cloning vectors allow first definitions of specific gene functions (Bono et al., 2000; Sartakova et al., 2000; Stewart et al., 2001). Concerning *ospC*, two recent studies based on genetically manipulated *B. burgdorferi* s.s. in the American vector *Ixodes scapularis* were contradictory. While in one study it was shown that *B. burgdorferi s.s.* requires OspC for infection of mice but not for dissemination to the tick salivary glands (Grimm et al., 2004), the other study showed that OspC facilitates the dissemination of the borreliae from the midgut to the salivary glands (Pal et al., 2004b).

In the present study, we wanted to elucidate the role of OspC for the European species *B. afzelii* in the European vector *Ixodes ricinus*. We previously have described a *B. afzelii* clone lacking the OspC protein because of a frame-shift mutation in the corresponding gene, which had lost its ability to disseminate from the midgut to the salivary glands of *I. ricinus* (Fingerle et al., 2000, 2002). After a positive preliminary test to transform *B. afzelii* with green fluorescent protein (GFP) as a confirmatory marker, we succeeded to complement the *ospC B. afzelii* mutant to subsequently investigate the borrelial dissemination behavior in capillary-fed *I. ricinus*. Our results presented herein indicate that OspC is required for dissemination of *B. afzelii* from the *I. ricinus* midgut to the salivary glands (Goettner et al., 2006).

Materials and methods

Ticks

All of the nymphal *I. ricinus* ticks used in this study were derived from a colony maintained at the Institute of Zoology, University of Neuchâtel, Neuchâtel, Switzerland. The laboratory-reared ticks had been free of *B. burgdorferi* s.l. infection for at least two generations. The nymphs were kept shaded at 10 °C and 95% relative humidity and were used not before 2 months post larval ecdysis at the earliest for the experiments.

Borrelia strains and clones

The OspC-negative *B. afzelii* clone PKo345 (cPKo345^{ospC-}) was derived by triple-colony selection of a reisolate from a joint of a gerbil, infected with low-passage *B. afzelii* strain PKo (a human skin isolate) (Fingerle et al., 2000; Wilske et al., 1988). As described previously, cPKo345^{ospC-} has an insertion of a guanine in the *ospC* gene at position 200, leading to a frame-shift mutation with a stop codon after position 222. This mutant does not produce OspC (Fingerle et al., 2000). A low-passage *B. afzelii* PKo wild-type strain producing OspC in culture served as positive control.

Strains and clones were cultured as previously described to a density of 10^7/ml in MKP medium at 33 °C (Preac-Mursic et al., 1991). For cultivation of the transformed cPKo345^{ospC+}, 50–200 μg/ml kanamycin was added to the media.

PCR, construction of pBSV2 derivatives, and transformation of *Escherichia coli*

Extraction of DNA of the *B. afzelii* wild-type strain PKo was performed with the High Pure Template Preparation Kit (Roche; Penzberg, Germany) according to the manufacturer's instructions and was used as template for PCR. o*spC* was amplified using standard primers from our laboratory as described previously with additional NdeI and HindIII restriction sites (underlined) (Schulte-Spechtel et al., 2006): *ospC-for* (5′-TAGTAGCATATGAAAAAGAATACATTAAG-TGCG-3′) and *ospC-rev* (5′-TAGGAGAAGCTTTTA-AGGTTTTTTTGGACTTTCTGC-3′).

The *gfp* gene was amplified from the pcDNA3.1/CT-GFP-TOPO vector (Invitrogen, Carlsbad, CA, USA) with the following primers (NdeI and HindIII restriction sites underlined): *gfp-for* (5′-TAGTAGCATATG-CGTAAAGGAGAAGAACTTTTC-3′) and *gfp-rev*