PREPARATION AND PURIFICATION OF RECOMBINANT OUTER SURFACE PROTEIN A (rOspA) OF *BORRELIA BURGDORFERI* SENSU STRICTO AND *BORRELIA AFZELII*

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The recombinant Outer surface protein A (rOspA) from *Borrelia burgdorferi* is a possible immunogen for protection of infected humans and animals against development of Lyme borreliosis (Lyme disease), a chronic tick-borne disease characterised by diverse dermatologic, neurologic, rheumatic, and cardiac manifestations. For several years, research and development have been directed towards a vaccine for the prevention of this debilitating disease. Numerous animal studies demonstrate that pre-existing antibodies against the outer surface proteins of *B. burgdorferi* can prevent infection and disease caused by this organism. In this communication, using recombinant DNA technology, genes from *B. burgdorferi* sensu stricto and *B. afzelii* were inserted into *E. coli*-expression vectors and the rOspA were produced. Our aim was to obtain rOspA protein in a purity and quantity desirable for immunization of experimental animals. rOspA is currently the most developed, molecularly-defined vaccine candidate for the prevention of Lyme borreliosis.

INTRODUCTION

Lyme borreliosis is a multisystem inflammatory disease caused by *Borrelia burgdorferi* a gram-negative spirochete¹, transmitted by the bite of infected ixodes ticks. *B. burgdorferi* sensu lato has been divided in to 11 genospecies. Of these, three species are recognized as pathogenic for humans: all of which are present in Europe: *B. burgdorferi* sensu stricto, the only species causing Lyme borreliosis in the United States; *B. afzelii*; and *B. garinii*. These spirochetes alternate in nature between warm-blooded hosts (mammals) and poikilothermic vectors. The tick *Ixodes ricinus* is the main vector of these pathogens². In the vector, the spirochetes replicate during the feeding process³, migrate through the gut wall, and invade various tissues, including the salivary glands, from where they are transmitted to the host blood via saliva⁴. OspA is abundantly expressed in unfed ticks, probably mediating adherence to midgut cells⁵ and thus enabling borreliae to survive in the vector for prolonged periods without tick feeding. *B. burgdorferi* expresses OspA but not OspC when residing in the midgut of unfed ticks. However, when the tick starts feeding on mammals, OspC synthesis is induced and OspA is repressed⁶. The switch is in part due to the change in temperature⁷.

Outer surface protein A (OspA) (molecular weight 31 kDa), a species-specific surface lipoprotein that presents together with the flagellin approximately one

third of the total spirochete protein. The crystal structure of OspA consists of a single-layer beta-sheet connecting N-terminal and C-terminal globular domains. The central beta-sheet consists largely of polar amino acids that are solvent-exposed on both faces; this seems to be unique among protein structures⁸. More than 100 other proteins have also been identified in the spirochete's genome, including OspB, OspC, OspD, OspE, and OspF (ref.⁹).

The OspA protein has been used as most promising candidate for vaccine against infection with *B. burgdorferi*. The ospA gene of *B. burgdorferi* encodes an outer membrane protein which is a major antigen of the Lyme disease agent. OspA elicits immunity against *B. burgdorferi* infection and is being used as a human vaccine¹⁰. OspA antibody blocks spirochete transmission to the vertebrate host by binding to *B. burgdorferi* within the gut of engorging ixodes ticks¹¹.

Active immunization studies with OspA immunogens shown high-level of protection in mice. Humoral immunity is sufficient for protection. Although binding to an antigenic determinant (conformational epitope) located within the carboxyl end of OspA appears to be important for protection, the mechanism of protection conferred by antibodies has not been established. A principal drawback of the vaccine is the heterogeneity of OspA protein among particular *B. burgdorferi* isolates¹².

At present, two human Lyme borreliosis vaccines use recombinant OspA (rOspA) from *B. burgdorferi* sensu

Fig. 1. **a)** SDS-PAGE of rOspA proteins from *B. burgdorferi sensu stricto* stained with Coomassie Blue and **b)** Western immunoblot developed with Penta-His HRP antibodies. Lane 1, High-Range Rainbow Molecular Weight Markers (Amersham Biosciences); Lane 2, flow-through fraction; Lanes 3 and 4, wash fractions; Lanes 5, 6, 7 and 8, elution fractions.

Fig. 2. **a)** SDS-PAGE of rOspA proteins from *B. afzelii* stained with Coomassie Blue and **b)** Western immunoblot developed with Penta-His HRP antibodies. Lane 1, High-Range Rainbow Molecular Weight Markers (Amersham Biosciences); Lane 2, flow-through fraction; Lanes 3 and 4, wash fractions; Lanes 5, 6, 7 and 8, elution fractions.

stricto. LYMERix, produced by SmithKline Beecham, and licensed for use in Canada, contains 30 µg of purified lipidated-rOspA protein combined with 0.5 mg of aluminium adjuvant¹³. The vaccine stimulates host immune system to produce OspA – specific antibodies. Studies suggest that when a tick bites a vaccinee, the antibodies enter and contribute to killing the bacteria in the tick. A second vaccine, ImuLyme, produced by Pasteur Merieux Connaught, contains 30 µg of purified lipidated-rOspA protein without adjuvant. The ImuLyme vaccine induces killing of the spirochetes within the tick before it can enter the human bloodstream¹⁴.

MATERIALS AND METHODS

Plasmid DNA was isolated from *B. burgdorferi sensu stricto* and *B. afzelii*. The DNA was used as a template for the PCR amplification of the *OspA* gene which was after purification (QIAEX II Gel Extraction kit, Qiagen) cloned into expression vector pDESTTM 17 (Invitrogen) using GatewayTM Technology (GT). His-tag-fused OspA protein was expressed in expression competent *E. coli* BL21 Star (DE3) (Invitrogen). BL21 Star (DE3) were grown for 12 h at 37°C in 2 liters of the LB medium un-

til they reached mid-log ($OD_{600} = 0.6$). IPTG (SERVA) was added at a final concentration of 0.1 mM, and the culture was incubated for another 4 h. Cells were collected by centrifugation (10.000 RPMI, 10 min., 4°C), resuspended in lysis buffer (lysis buffer: 50 mM $NaH_2PO_4 \cdot H_2O$, 300 mM NaCl, 10 mM imidazol, pH = 8) containing lysozyme (0.5 mg/ml) and incubated for 1 h at 4°C. After 10×10 s ultrasonic pulsions homogenisation lysate was centrifuged (10.000 RPMI, 10 min., 4°C) and 4 ml of the cleared supernatant was mixed with 1 ml of the 50% Ni-NTA slurry (Qiagen) for 1 h at 4°C. The supernatant – Ni-NTA mixture was loaded into a gravity column (Sigma) and washed twice with 4 ml washing buffer (washing buffer: 50 mM $NaH_2PO_4 \cdot H_2O$, 300 mM NaCl, 30 mM imidazol, pH = 8). Protein was eluted with 4× 0.5 ml of elution buffer (elution buffer: 50 mM $NaH_2PO_4 \cdot H_2O$, 300 mM NaCl, 250 mM imidazol, pH = 8). All buffers contained 5 mM β – mercaptoethanol, 0.5 mM PMSF, 2 µg/ml Leupeptin and 0.1 µg/ml Aprotinin. The fractions were analyzed using 12% SDS-PAGE and Western immunoblot. Total protein was stained on gel with Coomassie brilliant blue. Protein blotted on PVDF membranes were incubated with Penta-His HRP antibodies (Qiagen) and detected by a color reaction using TMB Membrane Substrate (Amresco).

Fractions with maximum concentration of rOspA were dialyzed against 200 mM NaCl at 4 °C overnight.

RESULTS

The rOspA proteins from *B. burgdorferi sensu stricto* and *B. afzelii* present 80 % of total protein in elution fractions and they were specifically recognized with Penta-His antibodies (Fig. 1, Fig. 2). The concentration of rOspA from *B. burgdorferi sensu stricto* (0.4 mg/ml) and *B. afzelii* (0.3 mg/ml) were measured using a densitometer.

Overdialyzed fractions with maximum concentration of rOspA were tested using polyclonal anti-OspA-IgG antibodies (by Bioveta, a.s.). The rOspA proteins reacted with this antibodies (data is not shown).

CONCLUSION

Purification of recombinant OspA from *B. burgdorferi sensu stricto* and *B. afzelii* by nickel-nitrilotriacetic acid (Ni-NTA) metal-affinity chromatography under native conditions is highly effective. Proof of the 'principle' that rOspA is a protective antigen (as a possible immunogen to protect against Lyme borreliosis) will be confirmed by active immunization experiments in mice.

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