DEVELOPMENT OF AN OspC VACCINE AGAINST LYME BORRELIOSIS

I. Livey and F. Dorner

ABSTRACT

Outer-surface protein C (OspC) is a plasmid-encoded lipoprotein and protective antigen produced by Lyme disease *Borrelia* species. OspC is potentially valuable as a vaccine component, but due to the high degree of antigenic heterogeneity of OspC, an OspC-based vaccine has to contain several antigenic forms of OspC. The production of a multivalent OspC vaccine using antigen expressed in *Borrelia burgdorferi* sensu lato is associated with many technical and economical disadvantages. Consequently, we have chosen to produce OspC in *Pichia pastoris* and to assess the ability of this recombinant OspC to induce antibody production and protective immunity. Mice were immunized with recombinant OspC combined with $Al(OH)_3$ and the antibody (IgG) response to OspC and the resistance of the immunized mice to infection with virulent *Borrelia afzelii* were evaluated. OspC produced in *Pichia pastoris* is highly immunogenic and protective when adsorbed to $Al(OH)_3$, an adjuvant that is acceptable for use in humans. Recombinant OspC derived from *Pichia pastoris* can be prepared in a form that is suitable for use in an OspC-based vaccine against Lyme Borreliosis.

KEY WORDS

Lyme Borreliosis, Borrelia, vaccine, OspC, Pichia pastoris

INTRODUCTION

A killed, whole-cell vaccine has been licensed for the prevention of Lyme Borreliosis (LB) in dogs (1). However, it is unlikely that a vaccine of this type will be developed for human use because of concerns about possible adverse reactions associated with certain antigens present in a whole-cell vaccine. To produce a safe and effective vaccine against LB for human use, the risk of potential side effects can be minimized by developing vaccines that contain only those components that are required for protection. Most attention has been focused on the development of sub-unit vaccines comprising purified *Borrelia* antigens produced using recombinant DNA technology. However, the expression of *Borrelia* antigens in the attenuated *Mycobacterium bovis* strain Bacille Calmette-Guerin (BCG) is another approach that is being pursued (2).

Two antigens of particular interest for use in candidate LB vaccines are the outer-surface proteins OspA and OspC. Both OspA (3,4) and OspC (5,6) are protective antigens in animal models of LB. In addition, both antigens are cell-surface exposed

lipoproteins (7,8), both are plasmid-encoded (8,9,10,11) and both are serologically heterogeneous (12). Despite these similarities, OspA and OspC may protect in very different ways. In unfed ticks infected with *Borrelia burgdorferi* (*Bb*) sensu stricto, the Lyme disease spirochaetes express OspA but no OspC (13). Upon feeding, there is a switch from OspA expression to expression of OspC, which is accompanied by the migration of spirochaetes from the tick mid-gut to the salivary glands where they can be transmitted to the vertebrate host. *Borrelia* can be eradicated from infected ticks feeding upon mice immunized with OspA due to the borreliacidal activity of antibodies to OspA (14). This killing of the spirochaetes in the tick prevents their transmission to the mammalian host. Less is known about the mechanism by which OspC protects but it is likely that the mode of protection is more conventional. Unlike OspA, OspC is clearly expressed during the early stages of LB since antibodies to OspC are one of the first serological indicators of infection (15). Protection conferred by OspC presumably occurs primarily in the mammalian host.

Sub-unit vaccines containing purified recombinant OspA, expressed in *E. coli*, have been tested in human volunteers and it appears that an OspA vaccine is both safe and immunogenic (16,17). Clinical trials to assess the protective efficacy of these vaccines are currently in progress. Both of these candidate OspA vaccines are monovalent, containing only one serological form of OspA. However, seven major serotypic forms of OspA have been described (18). It is questionable whether a monovalent OspA vaccine would be effective in Europe, where all seven serotypic forms of OspA are known to exist. However, in North America the chances that these monovalent OspA vaccines will be protective are better. LB in North America is associated with strains of *Bb* sensu stricto that express the serotype 1 OspA included in these vaccines.

We are endeavouring to develop a sub-unit vaccine containing OspC for the prevention of LB. Since OspC is highly polymorphic, it seemed to us very likely that an OspC-based vaccine would need to be multivalent in order to achieve a broad protection against a wide variety of LB isolates. Consequently, it was of great importance to characterize and classify the variability of OspC as a prerequisite to assessing the optimal mixture of serologically distinct forms of OspC that might be needed to give broad protection. A method of characterizing OspC heterogeneity using restriction fragment length polymorphism (RFLP) analysis of the *ospC* genes was developed for this purpose and used to analyze the *ospC* genes from a large collection of *Bb* sensu lato isolates (19). OspC variability is characterized by large numbers of polymorphic alleles and extensive sequence divergence between OspC variants. In light of the extreme variability observed for OspC, it is of considerable importance for the development of an OspC-based vaccine to assess whether OspC-mediated, protective immunity is highly type-specific or whether cross-protection between different forms of OspC is feasible. Preliminary results suggest that, although type-specific immunity is important for protection, immunity between different OspC types is possible (20). This suggests that protective immunity against Lyme disease *Borrelia* expressing a broad range of OspC types may be achieved by vaccination with a few selected OspC types. The production of a multivalent OspC vaccine would be facilitated by the use of recombinant DNA technology.

MATERIALS AND METHODS

Three groups of 10 female CD-1 mice (5-6 weeks old) were immunized subcutaneously with 200µl of purified, recombinant OspC (0.3, 1.0 or 3mg protein) adjuvanted with 2% $Al(OH)_3$. Three weeks later, the mice were re-immunized with the same material used in the primary immunization. The forty control mice remained untreated. Three weeks after the booster immunization, blood was taken so that OspC antibody titres could be determined and the mice were challenged intraperitoneally with the virulent *B. afzelii* strain König. Immunized mice received 10^4 spirochaetes and the challenge organism was titrated out in the control mice (i.e. 10^4, 10^3, 10^2, 10^1) to determine the infectious dose 50 of the challenge. Three weeks after challenging, blood was taken and 1:50 diluted plasma analyzed by western blotting to determine which mice had developed antibodies (IgM and IgG) to the infecting organism (excluding antibodies to the OspC immunogen). Mice that had sero-converted were scored as infected.

The OspC IgG antibody titre was measured in a ELISA using recombinant OspC (RFLP-type 8) as the coating antigen. Antibody preparations were titrated out and the titre expressed as the reciprocal of the dilution giving the same optical extinction as an OspC specific monoclonal antibody standard.

RESULTS AND DISCUSSION

With the aim of developing a recombinant sub-unit vaccine based on OspC, *ospC* genes from *Bb*

Table. Immunogenicity and protective efficacy of OspC produced in Pichia pastoris when CD-1 mice were immunized twice with 0.3, 1.0 or 3.0mg amounts of OspC adjuvanted with Al(OH)$_3$.

Immunogen (µg OspC)	Anti-OspC IgG Titre	Infected Mice Number Tested
0	<1,000	10/10
0.3	42,000	4/10
1.0	69,000	2/10
3.0	120,000	0/10

sensu lato strains were amplified by PCR, cloned into the episomal vector pHIL-A1, and the recombinant plasmid was transformed into the methylotrophic yeast *Pichia pastoris*. OspC was expressed in the cytoplasm of the yeast cells under the control of the methanol-inducible AOX1 promoter. The recombinant protein could be stably expressed with excellent yields of up to 700 mg OspC/l of culture being obtainable. The production of recombinant OspC in this yeast expression system offers several additional benefits. *P. pastoris* can be grown easily and economically in an inexpensive, chemically-defined medium in large bioreactors and does not have the complex nutritional requirements of the slowly growing *Bb* sensu stricto strains, which require an expensive, undefined medium containing proteins of animal origin. Producing OspC using recombinant DNA technology also guarantees that the product is free from the trace amounts of contaminating *Borrelia* antigens, which are inevitably present in a conventionally prepared product derived from *Borrelia*. The use of *P. pastoris* as a host for producing foreign antigens for pharmaceutical applications has the advantage over the commonly used *E. coli* in lacking endotoxin and this facilitates the manufacture of a pyrogen free product.

The immunogenicity and protective potency of OspC produced in *P. pastoris* were tested in mice. The animals were immunized twice, at a three week interval, with small doses of purified, recombinant RFLP-type 8 OspC (19), adjuvanted with Al(OH)$_3$, as shown in the Table. Three weeks after the booster immunization, the mice were challenged with the virulent *B. afzelii* strain König, which expresses OspC RFLP-type 8. Blood was taken shortly before challenge so that the OspC antibody titre at this time point could be evaluated. The mice responded to the recombinant OspC with a strong IgG antibody response as assessed in an OspC ELISA. The titre achieved with the smallest dose of antigen used (0.3mg) was higher than the highest OspC titres produced in experimentally infected mice. Titres increased with increasing doses of the immunizing antigen. The double immunization with 3mg of OspC protected all ten mice against a challenge with 10,000 spirochaetes, which was equivalent to 500 times the dose needed to infect 50% of unimmunized control mice; from the groups of ten control mice challenged with 10^4, 10^3, 10^2, 10^1 spirochaetes, 10, 10, 9 and 3 mice were infected respectively. With the smaller doses of OspC, the immune response afforded protection to most of the mice but was clearly insufficient to protect all mice against this high challenge dose.

These data demonstrate that recombinant OspC produced in *P. pastoris* is highly immunogenic and protective in small doses when combined with Al(OH)$_3$ as adjuvant, an adjuvant acceptable for use in humans. This recombinant form of OspC is clearly suitable for use in an OspC-based vaccine against LB.

REFERENCES

1. Chu HJ, Chavez LG, Blumer BM et al. Immunogenicity and efficacy study of a commercial *Borrelia burgdorferi* bacterin. J Am Vet Med Assoc 1992; 201: 403-11.

2. Stover CK, Bansal GP, Langerman S, Hanson MS. Protective immunity elicited by rBCG vaccines. In: Brown F (ed.). Recombinant vectors in vaccine development. Basel; Karger 1994; 82: 163-70.

3. Fikrig E, Barthold SW, Kantor FS, Flavell RA. Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. Science 1990; 250: 553-56.

4. Schaible UE, Kramer MD, Eichmann K et al. Monoclonal antibodies specific for the outer surface protein A (OspA) of *Borrelia burgdorferi* prevent Lyme Borreliosis in severe combined immunodeficiency (scid) mice. Proc Natl Acad Sci USA 1990; 87: 3768-72.

5. Preac-Mursic V, Wilske B, Patsouris E et al. Active immunization with pC protein of *Borrelia burgdorferi* protects gerbils against *Borrelia burgdorferi* infection. Infection 1992; 20: 342-49.

6. Probert WS, Lefebvre RB. Protection of C3H/HeN mice from challenge with *Borrelia burgdorferi* through active immunization with OspA, OspB, or OspC, but not with OspD or the 83-kilodalton antigen. Infect Immun 1994; 62: 1920-26.

7. Howe TR, Mayer LW, Barbour AG. A single recombinant plasmid expressing two major outer surface proteins of the Lyme disease spirochete. Science 1985; 227: 645-46.

8. Bergstrom S, Bundoc VG, Barbour AG. Molecular analysis of linear plasmid-encoded major surface proteins, OspA and OspB, of the Lyme disease spirochete *Borrelia burgdorferi*. Mol Microbiol 1989; 3: 479-86.

9. Barbour AG, Garon CF. Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends. Science 1987; 237: 409-11.

10. Marconi RT, Samuels DS, Garon CF. Transcriptional analyses and mapping of the *ospC* gene in Lyme disease spirochetes. J Bacteriol 1993; 175: 926-32.

11. Sadziene A, Wilske B, Ferdows MS, Barbour AG. The cryptic *ospC* gene of *Borrelia burgdorferi* B31 is located on a circular plasmid. Infect Immun 1993; 61: 2192-95.

12. Wilske B, Preac-Mursic V, Schierz G et al. Detection of IgM- and IgG antibodies to *Borrelia burgdorferi* using different strains as antigen. Zbl Bakt Suppl 1989; 18: 299-309.

13. Schwan TG, Piesman J, Golde WT et al. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. Proc Natl Acad Sci USA 1995; 92: 2909-13.

14. Fikrig E, Telford SR, Barthold SW et al. Elimination of *Borrelia burgdorferi* from vector ticks feeding on OspA-immunized mice. Proc Natl Acad Sci USA 1992; 89: 5418-21.

15. Padula SJ, Dias F, Sampieri A et al. Use of recombinant OspC from *Borrelia burgdorferi* for serodiagnosis of early Lyme disease. J Clin Microbiol 1994; 32: 1733-38.

16. Keller D, Koster FT, Marks DH et al. Safety and immunogenicity of a recombinant outer surface protein A Lyme vaccine. JAMA 1994; 271:1764-68.

17. Van Hoecke C, De Grave D, Lebacq E. Evaluation of three formulations of a candidate vaccine against Lyme disease in healthy adult human volunteers. In: Cevenini R, Sambri V, La Placa M (eds.). Advances in Lyme Borreliosis Research. Bologna; Societa Editrice Esculapio S.r.l. 1994; 123-26.

18. Wilske B, Preac-Mursic V, Goebel UB et al. An OspA serotyping system for *Borrelia burgdorferi* based on reactivity with monoclonal antibodies and OspA sequence analysis. J Clin Microbiol 1993; 31: 340-50.

19. Livey I, Gibbs CP, Schuster R et al. Evidence for lateral transfer and recombination in OspC variation in Lyme disease *Borrelia*. Mol Microbiol 1995; 18: 257-69.

20. Livey I, Dorner F. Entwicklung eines Impfstoffes gegen Lyme-Borreliose. In: Süss J (ed.). Durch Zecken übertragbare Erkrankungen FSME und Lyme-Borreliose. Weller Verlag 1995; 147-54.

AUTHORS' ADDRESSES

Ian Livey, PhD, Immuno AG, Biomedical Research Center, Uferstraße 15, Orth an der Donau, A-2304 Austria

Friedrich Dorner, PhD, professor, same address