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Identification of a defined linear epitope in the OspA protein of the Lyme disease spirochetes that elicits bactericidal antibody responses: Implications for vaccine development

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ABSTRACT

The lipoprotein OspA is produced by the Lyme disease spirochetes primarily in unfed ticks. OspA production is down-regulated by the blood meal and it is not produced in mammals except for possible transient production during late stage infection in patients with Lyme arthritis. Vaccination with OspA elicits antibody (Ab) that can target spirochetes in the tick midgut during feeding and inhibit transmission to mammals. OspA was the primary component of the human LYMErix™ vaccine. LYMErix™ was available from 1998 to 2002 but then pulled from the market due to declining sales as a result of unsubstantiated concerns about vaccination induced adverse events and poor efficacy. It was postulated that a segment of OspA that shares sequence similarity with a region in human LFA-1 and may trigger putative autoimmune events. While evidence supporting such a link has not been demonstrated, most efforts to move forward with OspA as a vaccine component have sought to eliminate this region of concern. Here we identify an OspA linear epitope localized within OspA amino acid residues 221–240 ($OspA_{221–240}$) that lacks the OspA region suggested to elicit autoimmunity. A peptide consisting of residues 221–240 was immunogenic in mice. Ab raised against $OspA_{221–240}$ peptide surface labeled *B. burgdorferi* in IFAs and displayed potent Ab mediated-complement dependent bactericidal activity. BLAST analyses identified several variants of $OspA_{221–240}$ and a closely related sequence in OspB. It is our hypothesis that integration of the $OspA_{221–240}$ epitope into a multivalent-OspC based chimeric epitope based vaccine antigen (chimeritope) could result in a subunit vaccine that protects against Lyme disease through synergistic mechanisms.

1. Introduction

Lyme disease, the most common tick-borne disease in the Northern Hemisphere, occurs in North America, Europe and Asia. The CDC estimates that there are at least 300,000 cases of human Lyme disease per year in the United States (http://www.cdc.gov). Lyme disease is also a significant health concern in veterinary medicine particularly in canines and equines [1,2]. In 2015, over 250,000 positive canine Lyme disease Ab tests were reported to the Companion Animal Parasite Council (CAPC; http://www.capc-vet.org/). Since only ~30% of test data are compiled, the actual number of Ab positive tests in canines is certainly much greater than 250,000 and may exceed 800,000. The incidence of Lyme disease in equines is less clear as only a few studies have investigated this. Seropositivity rates in equines in some regions of the United States are as high as 20–35% [3,4] (Marconi, RT; unpublished data). Equine Lyme disease has been reported in N. America, S. America, Asia and Europe [5–8].

The absence of reliable diagnostic assays for early Lyme disease makes it difficult to obtain confirmative serology to support what is largely a clinical diagnosis. This coupled with ongoing debate about appropriate treatment strategies and the potential for debilitating late stage disease outcomes, prevention of Lyme disease through vaccination offers a cost effective approach for prevention. While there are several Lyme disease vaccines labeled for use in canines, vaccines labeled for use in humans or equines are not commercially available. LYMErix™, a lipidated Osp (outer surface protein) A based vaccine, was available for human use from 1998 to 2002 before being withdrawn from the market [9,10]. OspA is a linear plasmid (linear plasmid of 54 kb; lp54) encoded lipoprotein that is co-transcribed with OspB [11]. OspA and OspB produc-

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tion are regulated by environmental conditions and selectively produced by spirochetes in the midgut of unfed ticks [12–16]. When *Ixodes* ticks feed, *ospAB* transcription is halted [17–19] and there is no significant production of OspA and B in mammals. A possible exception is transient production in humans suffering from late stage Lyme arthritis [20,21]. While OspAB do not typically elicit Ab responses during natural infections, Ab elicited by vaccination with OspAB can elicit varying levels of protection. Anti-OspAB Ab can target spirochetes in the tick midgut during the blood meal and thereby inhibit transmission from ticks to mammals [22]. As expected for a transmission blocking vaccine that specifically targets a pathogen in its arthropod vector, protection is strictly dependent on circulating Ab titer. This dependency most likely explains the relatively low efficacy (73% efficacy) observed with LYMErix™ even after a three dose schedule delivered over 18 months (reviewed in [23]).

Recombinant non-lipidated OspA is also a core component of two subunit canine Lyme disease vaccines. VANGUARD®crLYME (ZoetisUS) consists of r-OspA and a unique r-OspC derived chimeric epitope based protein (chimeritope) [24–27] and RECOMBITEX® Lyme (Merial) consists of OspA alone. The limitations of OspA (discussed above) suggest that it alone is insufficient to effectively serve as primary component of future vaccine formulations [9,10,24,28–32]. In addition, earlier studies postulated that OspA residues 165-173 ($OspA_{165–173}$; IYVIEGTSKQDLTSF) elicit Ab that could be cross-reactive with human LFA-1 protein triggering vaccine induced arthritis and autoimmune reactions [33,34]. Several research groups have sought to generate modified OspA proteins or OspA chimerics that lack the epitope of putative concern [33,35–37]. In this study we sought to identify a means by which the positive protective effects of OspA can be exploited in the context of an epitope based chimeric protein. By incorporating an isolated OspA epitope into an OspC epitope based vaccine [25–27,38], hypothetical concerns about adverse events associated with the use of full length OspA can be eliminated.

As alluded to above, we have pursued the development of chimeric epitope based protein vaccines (chimeritopes) for tick-borne diseases [25–27]. Chimeritopes offer advantages over traditional subunit vaccines and protein chimeric vaccines. They can be designed to include a diverse array of linear epitopes derived from multiple variants of a protein and thus provide broad protective efficacy. Importantly, regions of a protein putatively associated with adverse events, such as $OspA_{165–173}$ region, can be omitted. The OspC based chimeritope included in the VANGUARD®crLyme canine vaccine consists of a series of linear epitopes (designated as the L5 and H5 epitopes) derived from multiple OspC types [25–27,39]. OspC is an attractive candidate for vaccine development because of it antigenic properties and expression patterns. OspC production is significantly upregulated by exposure to blood in ticks [15,40] and expression remains high during early stage infection in mammals [41]. The primary goal of this study was to identify a defined linear epitope of OspA for potential inclusion into an OspC chimeritope. A combined OspC/OspA chimeritope has the potential to convey protection through independent but potentially synergistic mechanisms: (1) Ab targeting of spirochetes in ticks to inhibit transmission [42,43], and (2) killing of spirochetes in mammals by α-OspC Ab [44]. In summary, an OspA linear epitope spanning residues 221–240 ($OspA_{221–240}$) was identified. Peptide corresponding to this region elicited Ab in mice that surface labeled *B. burgdorferi* and killed in an Ab mediated-complement dependent manner. This study supports the possible inclusion of the $OspA_{221–240}$ epitope variants in an OspC based chimeritope.

2. Materials and methods

2.1. Bacterial strains and bacterial cultivation

Borrelia isolates employed in the study are described in Table 1. All isolates were cultivated in BSK-H complete medium (Sigma-Aldrich) supplemented with 6% rabbit serum (37 °C, 5% CO_2). Growth was monitored by dark-field microscopy.

2.2. Protein production and purification

Recombinant full-length OspA (minus the leader peptide) and OspA subfragments (~50 aa subfragments with 25 aa overlaps) were generated by PCR amplification using *B. burgdorferi* B31 DNA as template [11] (GenBank: CAA32579.1). All PCR primers were deigned with tail sequences to facilitate ligase independent cloning (LIC) and annealing into pET-32 Ek/LIC (Novagen). Proteins produced with this vector possess an N-terminal S-Tag. All primer sequences are listed in Table 2 with the segment of the protein encoded by each amplicon indicated. PCR was performed using standard cycling conditions and *Pfu* polymerase (Promega). Amplicons were purified using PCR purification kits (Qiagen) and then annealed with linearized pET-32 Ek/LIC per the supplier's protocol (Novagen). The plasmids were propagated in *E. coli* NovaBlue (DE3) cells (Novagen), recovered using QiaFilter midi-plasmid purification kits (QIAGEN) and the inserts sequenced on a fee for service basis (MWG Biotech). For protein production, *E. coli* BL21 (DE3) cells were transformed with the recombinant pET-32 Ek/LIC plasmids and protein expression induced with 1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) using standard protocols. Cells

Table 1
Strain description and summary of bactericidal assays.

Species and strain description/OspA designation	% Killing with α-$OspA_{221–240}$	% Killing with α-$OspA_{full\ length}$	% Killing with α-KLH
B. burgdorferi B31Ml (OspA type a#1): clonal population derived from isolate B31 that was isolated from an *Ixodes scapularis* tick (USA) [55]	84.9 ± 3.73	97.6 ± 0.46	0
B. burgdorferi 2E6 (OspA type a#1): *ospA* gene inactivation mutant derived from the B31 clone 5A3 [47]	44.3 ± 1.8	100 ± 0	ND
B. burgdorferi 7A (OspA type a#1): *ospB* gene inactivation mutant derived from the B31 clone 5A3 [47]	100 ± 0	100 ± 0	ND
B. burgdorferi DRI40 h (OspA type a#1): clonal population derived from strain DRI40 that was recovered from a purpose bred beagle infected with field collected ticks (USA) [49]	84.0 ± 1.8	98.1 ± 1.7	ND
B. bavariensis PbaeII (OspA type a#7): cerebrospinal fluid of a human Lyme disease patient (Germany) [56]	70.0 ± 2.0	97.4 ± 2.1	ND
B. bavariensis Phoe (OspA type a#7): cerebrospinal fluid of a human Lyme disease patient (Germany) [56]	70.9 ± 3.9	100 ± 0	ND
B. afzelii J1 (OspA type #6): *I. persulacatus* tick (Japan) [57]	36.8 ± 4.3	98.3 ± 0.4	ND
B. afzelii B023 (OspA type unknown): skin biopsy from an erythema migrans lesion from a patient (Germany) [58]	27.9 ± 2.2	100 ± 0	ND
B. afzelii DK26: skin biopsy from an erythema migrans lesion from a patient (Denmark) [59]	15.3 ± 4.8	98.8 ± 1.1	ND

ND: not determined.

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