# Characterization of recombinant OspA in two different *Borrelia* vaccines with respect to immunological response and its relationship to functional parameters[1]

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## Key Points

- All Lyme vaccines utilize OspA to elicit an antibody-mediated immunity that prevents transmission of *Borrelia burgdorferi.*
- RECOMBITEK® Lyme is the only nonadjuvanted Lyme vaccine and it contains lipidated OspA that forms large (418 kDa, 18 nm) complexes.
- VANGUARD® crLyme is an adjuvanted vaccine and contains non-lipidated OspA that exists as small (34 kDa, 6.4 nm) monomers.
- Compared to VANGUARD® crLyme, RECOMBITEK Lyme produced an earlier onset, as well as significantly stronger OspA immune response and significantly higher borreliacidal activity.

## Introduction

- There are several USDA-approved vaccines for prevention of *Borrelia burgdorferi* infection in dogs. All of these vaccines utilize outer surface protein (OspA) which is a potent immunogen and has been demonstrated to stimulate antibody-mediated immunity. All OspA-based vaccines help prevent transmission of disease by neutralizing *B. burgdorferi* within the midgut of the tick vector. Several vaccines containing OspC in addition to OspA are purported to provide a second line of defense within the vertebrate host. However, supportive data for demonstration of durable, globally relevant, and independently effective OspC-mediated protection in a challenge-type study with dogs (and people) is still lacking. Most recently, it has been proposed that efficacy of monovalent OspA targeting vaccines is "approximately 72%,"[2] without citing the specific monovalent OspA vaccine in question. This omission may lead one to believe that all monovalent OspA vaccines perform with similar results, which is inconsistent with several studies using RECOMBITEK Lyme vaccine (monovalent OspA) showing not only 100% prevention of infection[3,4] but also significant prevention of *Borrelia*-associated histopatholgy.[5]
- In order to address some of the above-mentioned inconsistencies as well as evidence showing necessity of OspA for protection against *B. burgdorferi* transmission, the immunological response of dogs to OspA from two commercially available vaccines was compared: a nonadjuvanted, monovalent, recombinant OspA vaccine (here on noted as RECOMBITEK Lyme) and an alum-adjuvanted, recombinant OspA, chimeric OspC vaccine (here on noted as VANGUARD® crLyme). Serological responses to OspA as well as borreliacidal activity were examined. On finding that RECOMBITEK Lyme vaccine elicited a more robust immunological response than the VANGUARD® crLyme vaccine, further characterization of the OspA antigen of the two vaccines with respect to their biochemical and biophysical properties was conducted.

## Methods

- Twenty-one purpose-bred beagle dogs with no prior history of vaccination against Lyme disease were separated into three groups: vaccination with RECOMBITEK Lyme (n=9), VANGUARD® crLyme (n=9) or unvaccinated control (n=3). All treatment dogs were vaccinated on day 0 and day 21 in addition to a 5-month booster.* Blood samples were taken from all dogs prior to the 1st and 2nd vaccinations as well as on days 14, 21, 28, 56, 64 and 112 days following the 2nd vaccination and 14 days following the 5-month booster.

## Results:

- **Serological response:**
  **Assessment of anti-OspA antibody response:** All serum samples were assayed using a fluorescent bead-based multiplex assay for the simultaneous detection of antibody against *B. burgdorferi* OspA, OspC, and OspF (Multiplex by Cornell University Diagnostic Laboratory, Ithaca NY).
- Onset of anti-OspA antibody response: RECOMBITEK Lyme produced an early onset of anti-OspA antibody response by 18 days following a single vaccination. This response was significantly greater than that induced by VANGUARD® crLyme at that time.
- Strength of anti-OspA antibody response: RECOMBITEK Lyme induced an anti-OspA antibody response that was higher than VANGUARD® crLyme at all subsequent time points, but the difference was not statistically significant.

# Results (cont.):

- **Assessment of anti-OspA IgG antibody:** The concentration of total anti-OspA IgG as well as specific IgG1, IgG2, IgG3 and IgG4 subclasses were measured. IgG1 has been identified in dogs to be involved in antibody-dependent cell-mediated cytotoxicity, cell-mediated cytotoxicity and C1q complement binding.
- Total anti-OspA IgG: RECOMBITEK® Lyme produced significantly higher total IgG titers than VANGUARD® crLyme at each point – Day 18, Day 42, Day 105 and Day 175.
- Anti-OspA IgG subclasses: with exception of IgG3, all subclasses had significantly higher titers with RECOMBITEK Lyme than Vanguard® crLyme and IgG1 titers were more homogenous with RECOMBITEK Lyme than VANGUARD® crLyme.
- **Avidity:** Avidity is a measure of the accumulated strength of antibodies binding to their target antigen. Higher avidity antibodies are known to be more effective by increasing contact time with the antigen.
- RECOMBITEK Lyme produced a large amount of specific high-avidity antibodies, which increased after each vaccination and reached a maximum value after the last vaccination at 5 months. In contrast, VANGUARD® crLyme produced antibodies with much lower avidity that was not improved by the third vaccination at 5 months.
- RECOMBITEK Lyme produced significantly higher avidity than VANGUARD® crLyme on days 18, 105 and 175.

## Borreliacidal Activity:

- The ability of each vaccine to produce borreliacidal activity was assessed in culture using the serial dilutions of each study dog's serum
- RECOMBITEK Lyme produced consistently and significantly higher borreliacidal activity than VANGUARD® crLyme 18 days after 1st vaccination, 14 days after 2nd vaccination and 14 days after 5-month booster vaccination.

## Biochemical and Biophysical properties of OspA:

Natural spirochete-associated OspA is lipidated at the N-terminus of the amino acid sequence. Lipidation is acknowledged to be strongly immunostimulatory, both by itself and as a promoter of micelle formation.[6] The lipidation as well as molecular mass of the OspA in both vaccines was assessed using column chromatography and refractometry.

- RECOMBITEK Lyme was determined to be a mix of bi- and tri-lipidated forms with a molecular weight of 418 kDa and diameter of 18 nm.
- VANGUARD® crLyme was determined to be non-lipidated with a molecular weight of 34 kDa and diameter of 6.4 nm
- Differences in secondary confirmation (i.e., folding) or beta-sheet formation was ruled out, suggesting the size difference is due to the presence of lipid moieties.

# Conclusions:

- These results demonstrate that an earlier and higher level of anti-OspA antibody response as well as higher avidity anti-OspA antibodies are generated by RECOMBITEK Lyme than by VANGUARD® crLyme.
- These results also demonstrate that RECOMBITEK Lyme produced consistent and significantly higher borreliacidal activity than VANGUARD® crLyme.
- Lipidation of the OspA antigen present in RECOMBITEK Lyme is hypothesized to account for its superior immunogenic profile over the non-lipidated OspA antigen in VANGUARD® crLyme.

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* The 5-month booster was part of the study design and provided immunological data pertinent to this study.

**References**


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