Effects of OspA Vaccination on Lyme Disease Serologic Testing

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Received 7 May 1999/Returned for modification 15 July 1999/Accepted 11 August 1999

This study presents the effects of OspA vaccination on two-step testing for Borrelia burgdorferi antibodies. Although vaccinees developed enzyme-linked immunosorbent assay reactivity, immunoblots did not fulfill Centers for Disease Control and Prevention criteria for positivity. Furthermore, OspA reactivity did not interfere with interpretation of immunoblots with sera from patients who developed early Lyme disease despite vaccination.

In December 1998, the Food and Drug Administration approved a recombinant outer surface protein A (OspA) vaccine preparation for prevention of Lyme disease (2). It has been suggested that widespread use of this vaccine will affect the performance of serologic testing for Lyme disease because the OspA antigen is a component of the whole-cell sonicate of Borrelia burgdorferi, which is used in most commercially available enzyme-linked immunosorbent assays (ELISAs) (11). This study evaluates the potential impact of OspA vaccination on two-stage B. burgdorferi serologic testing.

Serum samples. Sequential serum specimens were obtained from 17 healthy adults participating in a phase 2 safety and immunogenicity study sponsored by Connaught Laboratories, Inc. (Swiftwater, Pa.). Serum specimens were collected prior to receipt of the first 30-μg intramuscular dose of the OspA vaccine (pre), at day 30, when a second 30-μg OspA vaccine dose was administered, and at days 60, 90, and 180 after the first vaccination. Serum samples were stored at −70°C until time of testing. Linkage was removed before testing.

To illustrate vaccine effects further, serum samples from selected individuals receiving the same OspA vaccine preparation in an efficacy study (12), including patients who developed early Lyme disease with erythema migrans despite vaccination, were studied. In the efficacy study, three 30-μg doses of vaccine were given to the volunteers at time 0, day 30, and 1 year.

ELISA. Specimens were tested in an immunoglobulin M (IgM)-IgG ELISA (Lyme Stat; Whittaker M. A. Bioproducts Inc., Walkersville, Md.) in accordance with the manufacturer’s instructions.

Immunoblots. Separate IgM and IgG immunoblots (MarDx Diagnostics, Inc., Carlsbad, Calif.) were used to test all the serum specimens according to the manufacturer’s instructions. The Centers for Disease Control and Prevention-Association of State and Territorial Public Health Laboratory Directors (CDC/ASTPHLD) criteria were used for blot interpretation (3).

None of the 17 volunteers enrolled in the phase 2 trial had a positive ELISA or immunoblot result prior to receiving vaccination. Nine (53%) had a positive ELISA by 30 days after receipt of the first 30-μg dose of the recombinant OspA vaccine preparation. Sixty days after receipt of the first vaccine dose, and all 17 (100%) were reactive by ELISA by 30 days following the second dose (day 60) (Table 1).

### TABLE 1. Reactivity by ELISA and IgM and IgG immunoblot tests after OspA vaccination in 17 individuals participating in phase 2 of the Connaught Lyme vaccine trial

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<thead>
<tr>
<th>Day after vaccination</th>
<th>% of samples with:</th>
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<tr>
<td></td>
<td>Positive ELISA</td>
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<tr>
<td>Pre</td>
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<td>30c</td>
<td>53</td>
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<td>60d</td>
<td>100</td>
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<tr>
<td>90</td>
<td>76</td>
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<td>180</td>
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The highest mean Lyme index value (LIV) by ELISA was observed at day 60 (Fig. 1).

Both IgM and IgG antibodies to OspA were observed by immunoblot tests of serum samples from 65% of vaccine recipients by day 30. While IgM reactivity to OspA at day 60 was nearly identical to that at day 30, the IgG reactivity to OspA had risen to 93%. By day 180, 88% still showed IgG reactivity to OspA whereas only 18% had IgM reactivity. Of interest, despite the high rate of OspA reactivity by IgG immunoblot on day 180, only 35% were still reactive by ELISA. Importantly, none of the IgM or IgG immunoblot reactivities at any time point fulfilled the CDC/ASTPHLD criteria for a positive immunoblot result (Fig. 2).

Although none of the immunoblots was interpreted as positive, several noteworthy effects on immunoblot reactivity were observed. In addition to the expected reactivity at the 31-kDa band (OspA), those serum samples with intense IgG OspA reactivity regularly had several bands of smaller molecular sizes, including bands of approximately 22 and 16 kDa, particularly in individuals receiving three vaccine doses (Fig. 3). The reason for the production of these additional bands is not known, but they may represent reactivity to degradation products of OspA or to other smaller-molecular-weight proteins which have amino acid sequences in common with epitopes of OspA. The presence of these bands might lead to diagnostic confusion if they are interpreted as indicating reactivity to the 18-kDa protein, which is a band of diagnostic importance for IgG immunoblot interpretation. Sera with OspA antibodies were also associated with darkening of the immunoblot extend-
Figure 5 shows examples of immunoblots of serum samples from participants in an efficacy study (12) who despite OspA vaccination developed Lyme disease. Although such patients generally had weak OspA reactivity, the bands of diagnostic significance on IgM immunoblots were readily visualized. In our laboratory, similar findings to those presented in this study were observed in serum samples from individuals receiving the Food and Drug Administration-approved Lyme disease vaccine manufactured by SmithKline Beecham (data not shown).

If OspA vaccination becomes widely used, current ELISA testing using _B. burgdorferi_ that contains OspA will become superfluous. Unfortunately, use of immunoblotting alone without a preceding ELISA will reduce specificity and increase costs. In studies to date, the reduction in specificity has ranged from 1.5 to 8% (mean, 4.1%), depending on the particular group of control sera tested and the particular ELISA and immunoblot tests used (4, 5, 9). Even a small reduction in specificity would have a substantial impact on the predictive value of a positive test when testing patients with a low pretest probability of Lyme disease (6).

Further, under the assumptions of an ELISA negativity rate of 75% in clinical practice (unpublished data) and a cost of $52 for an ELISA and $106 for an immunoblot test (8), an additional expenditure of 77 million dollars per year would be incurred by routinely performing immunoblot tests instead of conditional two-stage testing for the estimated 2.8 million serum samples submitted for Lyme disease serologic testing annually in the United States (10).

Pending the introduction of assays without OspA (13), we recommend that serologic testing for Lyme disease be limited to patients with at least a 20% pretest probability of Lyme disease (1) and that initial ELISA testing be omitted for OspA-immunized individuals.

We thank Eleanor Bramesco, Donna McKenna, Diane Holmgren, Susan Bittker, Denise Cooper, and Louis Rosenfeld for their assistance. We also thank Daniel Benevento for his expert photographic assistance.

REFERENCES


