NOTES

Evaluation of the C6 Peptide Enzyme-Linked Immunosorbent Assay for Individuals Vaccinated with the Recombinant OspA Vaccine

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The C6 enzyme-linked immunosorbent assay (ELISA), based on a peptide C6 that reproduces the sequence of invariable region 6 of VlsE, the antigenic variation protein of Borrelia burgdorferi, has been shown to be a sensitive and specific test for the serologic diagnosis of Lyme disease. We now report that none of 29 uninfected individuals vaccinated with the recombinant OspA vaccine had an antibody response to the C6 peptide. The C6 peptide ELISA can be used to diagnose Lyme disease in patients who have received the OspA vaccine.

Lyme disease (LD) is the most common vector-borne illness in the United States, and it is also endemic in Europe and parts of Asia. LD is caused by a group of genetically diverse spirochetes collectively termed Borrelia burgdorferi sensu lato. The diagnosis of LD is based on the patient’s clinical history and physical findings, and depending on the stage of the disease, the diagnosis is corroborated by laboratory evidence of infection (11). Serologic testing is the most commonly used corroborative test in both Europe and the United States. Conventional assays use whole-cell lysates of cultured spirochetes as the antigen. Due to the presence of cross-reactive antigens shared between B. burgdorferi and other bacteria, these assays can have low specificities (4). There are also problems with inter- and intralaboratory variabilities of the assays (2). Due to these difficulties, a two-tiered testing strategy for the serodiagnosis of LD has been recommended by the Centers for Disease Control and Prevention (CDC) since 1995 (3). This strategy consists of the use of an enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay (IFA), followed by Western blotting when the results of the first tier of tests are indeterminate or positive.

The use of the recombinant outer surface protein A (rOspA) vaccine (LYMErix; GlaxoSmithKline, Research Triangle Park, N.C.) has compounded the problem of the serodiagnosis of B. burgdorferi. Physicians can face difficulties in using whole-cell antigen-based tests to help in the diagnosis of B. burgdorferi infection in patients who have received the rOspA vaccine, as the antigen extracts used at present include OspA (1, 5, 10). Although use of this vaccine has recently been discontinued, there are several reasons why a diagnostic test that circumvents detection of anti-OspA antibodies is still relevant. First, more than 1.4 million doses of LYMErix have been sold (LYMErix Lyme disease vaccine [recombinant OspA] briefing document for Vaccines and Related Biological Products Advisory Committee, January 31, 2001, GlaxoSmithKline [http://www.fda.gov/ohrms/dockets/ac/01/slides/3680s2.htm]), and thus, one may estimate that about one-half million people have received the vaccine regimen. Second, the antibody response to the vaccine can last at least 12 months after receipt of the third dose (13), and the length of the antibody response in an individual patient is unknown; hence, physicians can still face difficulties when evaluating such patients. Third, the OspA vaccine development program has not been discontinued in Europe, where the C6 peptide test may be used (6), and future vaccine formulations to be used in the United States might still contain OspA.

Western blotting has been recommended as the preferred test for evaluation of vaccinated subjects for possible B. burgdorferi infections, but interpretation of the blots can also be complicated by the presence of anti-OspA antibodies. Immunoblots for subjects who have received the rOspA vaccine can show several bands of smaller molecular sizes, in addition to the expected reactivity in the 30- to 31-kDa region (OspA). The presence of these bands can lead to confusion in making a diagnosis. OspA antibodies can also cause darkening of the immunoblot region that extends above the 30- to 31-kDa band, with the ensuing difficulty in assessing specific bands in that area (1, 10). In some immunoblots, multiple reactive bands can appear (5, 10).

A procedure that is intrinsically unable to detect anti-OspA antibodies could, in principle, circumvent these difficulties for individuals who have received the rOspA vaccine. The use of a B. burgdorferi sensu stricto strain that lacks the plasmid encoding OspA and OspB eliminated reactivities with anti-OspA antibodies by both ELISA and immunoblotting (10, 14, 16), but additional studies are required to demonstrate the sensi-
tivities and specificities of tests with this strain compared with those of the tests used at present. In one study, both an ELISA with this strain and a full-length recombinant OspC protein ELISA had poor specificities (14).

We recently developed a peptide-based ELISA using as the antigen a 26-mer synthetic peptide (the C6 peptide) based on the invariant region 6 (IR6) of the VlsE (Vmp-like sequence, expressed) lipoprotein of *B. burgdorferi* (9). IR6 remains unchanged during antigenic variation and is both structurally and antigenically conserved among pathogenic *B. burgdorferi* strains and genospecies (7, 15). IR6 is highly immunogenic; all experimentally infected animals tested (including mice, monkeys, and dogs) produce early, persistent, and strong antibody responses to this sequence (7-9). When the C6 peptide was used in a diagnostic ELISA with serum samples from U.S. patients, the assay had excellent sensitivities with acute-, convalescent-, and late-phase specimens (74, 85 to 90, and 100%, respectively). It also had excellent specificity and precision (99 and 100%, respectively) (9). This test is now approved by the Food and Drug Administration for use for the diagnosis of LD. Also, because of the antigenic conservation of the IR6 region among *B. burgdorferi* sensu lato strains, the C6 peptide ELISA was able to detect antibody in patients that had culture-confirmed infections caused by either *Borrelia garinii* or *Borrelia afzelii*, the two genospecies most prevalent in Europe (6).

An important advantage of the C6 peptide ELISA would be its predictable lack of cross-reactivity with anti-OspA antibodies. Preliminary data from studies with animals have supported this notion, as the C6 peptide ELISA did not detect anti-OspA antibodies in the sera of four monkeys or five dogs vaccinated with the rOspA vaccine (8, 9). In the present study, we show that there is no cross-reactivity with antibodies elicited by vaccination with rOspA in humans. Therefore, the C6 peptide ELISA can be used for the diagnosis of LD in patients who have received this vaccine.

Serum samples were collected from 29 volunteers who had received at least two doses of the rOspA vaccine (LYMErix; GlaxoSmithKline) and who had no history of LD. Samples from five patients with LD (two patients with Lyme arthritis, one patient with late neuroborreliosis, one patient with localized erythema migrans, and one patient with disseminated erythema migrans) and samples from five healthy volunteers who had no history of LD and who had not received the rOspA vaccine were used as controls. All patients and volunteers gave written informed consent to participate in the study, and the study was approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases.

Serum samples were evaluated by the C6 peptide ELISA, which was performed according to the instructions of the manufacturer (Immunetics, Inc., Cambridge, Mass.); an OspA ELISA, which was performed as described previously (12); and a *B. burgdorferi* whole-cell lysate immunoglobulin G (IgG) ELISA, which was performed according to the instructions of the manufacturer (MarDx; Trinity Biotech, Carlsbad, Calif.). The investigator performing the tests was blinded to the sample source (vaccinee, patient, or healthy volunteer). Samples were also evaluated at a national reference laboratory by a commercially available *B. burgdorferi* whole-cell lysate IgG and IgM ELISA (bioMerieux, Hazelwood, Mo.), an IgM IFA, and a Western blotting assay for *B. burgdorferi* IgG (MarDx).

The results are shown in Table 1. All of the serum specimens from the 29 volunteers vaccinated with rOspA were negative by the C6 peptide ELISA. In contrast, only 10 samples were negative by the standard *B. burgdorferi* whole-cell lysate ELISA. When we measured the anti-OspA responses, only four of the vaccinees were negative by the OspA ELISA. Samples from 23 of 29 vaccinees were positive by the *B. burgdorferi* ELISA conducted at the reference laboratory. There was 76% concordance between the results of both whole-cell lysate ELISAs applied to the samples from vaccinees. The reference laboratory performed Western blotting with samples from all 23 vaccinees with a positive ELISA result. All of the Western blotting results were negative when the CDC criteria for a positive IgG Western blotting result were used. Two samples each were read as having four and three positive bands, and seven samples were read as having two positive bands. All samples tested were positive for the 30-kDa band (as expected), followed by the 41-kDa band (for which eight samples were positive). Other bands for which samples were positive included bands of 18 kDa (one sample), 23 kDa (three samples), 28 kDa (two samples), and 39 kDa (one sample). The immunoblotting results for 10 of the samples were uninterpretable due to the vaccine-induced antibody response.

The specimens from healthy volunteers were negative by all of the tests. All samples from patients with LD were positive by the C6 peptide ELISA. They were also positive by whole-cell lysate ELISAs. All except one of the patients had a positive IgG Western blotting result when the criteria of CDC were used. The patient with localized erythema migrans had a positive IgM IFA result. Only two patients had positive antibody responses against OspA.

Our data confirm that the C6 peptide ELISA is very specific for *B. burgdorferi* and that it does not detect anti-OspA antibodies in human samples. Because of its simplicity and high degrees of sensitivity, specificity, and precision, this test will be helpful in alleviating some of the remaining problems with the serodiagnosis of LD.

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## REFERENCES
