Brief Definitive Report

Protection against Antigenically Variable *Borrelia burgdorferi* Conferred by Recombinant Vaccines

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Summary

Due to local variation in the antigenicity of the agent of Lyme disease (*Borrelia burgdorferi*), a vaccine derived from any one isolate of this spirochete may fail to protect against the heterogeneous population of organisms that may be present in an enzootic focus. Accordingly, we determined whether antigenically variable spirochetes delivered by naturally infected ticks, collected from a site where transmission is intense, may fail to infect mice actively immunized with recombinant glutathione transferase outer surface fusion proteins A or B (OspA and OspB). Virtually all mice vaccinated by either immunogen appeared not to become infected, as determined by culture or histopathology of their tissues. We conclude that Osp vaccination of mice effectively prevents infection by the agent of Lyme disease in a simulated natural cycle of transmission.

The outer surface proteins (OspA and OspB) of the spirochetal agent of Lyme disease (*Borrelia burgdorferi*) hold promise as immunogens for delivery in vaccines designed to reduce risk of human Lyme disease (1, 2). Mice actively immunized with OspA cloned from the N40 strain of spirochetes (herein designated OspA-N40) are protected against a syringe challenge by the same spirochetal isolate. Recombinant OspB antigen cloned from strain B31 (OspB-B31) (3) similarly protects mice against homologous syringe challenge. Vaccination by recombinant OspA-N40 also protects against homologous challenge via the bites of nymphal deer ticks (*Ixodes dammini*) (4).

Isolates of the Lyme disease spirochete frequently differ antigenically, even when taken from the same site (5–7). Variability mainly affects the Osps and is particularly evident in European isolates (8, 9). Indeed, vaccination with OspA-N40 did not protect mice against syringe challenge by heterologous spirochetes (strain 25015) in which the OspA differs by 40 amino acids (10). Similarly, vaccination with OspB-B31 did not protect against challenge with N40 spirochetes (3). Because protective immunity may be strain or site specific (11), these laboratory studies suggest that a monotypic recombinant antigen may have limited value as a vaccine.

Although the OspA-N40 vaccine effectively protects mice against experimental homologous tick-borne spirochetal infection (4), naturally occurring spirochetal variants may evade this induced immunity. To examine this suggestion, we permitted nymphal deer ticks taken from vegetation in an intensely enzootic site to engorge upon mice treated with recombinant OspA or OspB immunogen and determined whether vaccination effectively neutralizes these naturally derived spirochetes.

Materials and Methods

Nymphal and adult deer ticks were collected by dragging from vegetation on the property of the University of Massachusetts Nantucket Field Station (Nantucket, MA) during June 1992. This site has previously been described (12). About 40% of host-seeking nymphs taken there contain Lyme disease spirochetes. Between 50 and 80 cases of Lyme disease are diagnosed each summer at the local hospital (S. R. Telford III, unpublished observations). Thus, this site is taken to represent the general venue of Lyme disease transmission in the northeastern United States.

Recombinant Osp's were expressed and purified as a fusion protein with glutathione transferase as previously described (1). Briefly, the gene for OspA-N40 was ligated into plasmid pGEX-2T, and used to transform *Escherichia coli*. Fusion protein production was induced with isopropyl β D-thiogalactopyranoside (IPTG), and purified using a glutathione sepharose 4B column. Female, 3–4-wk-old C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME) were actively immunized with 10 μg of recombinant OspA or OspB fusion proteins in CFA, and boosted with the same amount of pro-

[1] S. R. Telford and E. Fikrig made equivalent contributions to this work.

tein in IFA on days 7 and 14. For comparison, the glutathione transferase carrier was similarly injected into other mice.

To challenge immunized mice, five field-derived nymphal ticks were permitted to attach to each host 4 wk after the series of immunizations was completed. All ticks were permitted to feed to repletion and to detach naturally over water. Engorged ticks were retained for 10 d at room temperature, at which time they were examined for the presence of spirochetes by means of darkfield microscopy and indirect immunofluorescence using polyclonal or monoclonal antibodies to *B. burgdorferi*, as described (4). In addition, lysates from randomly selected ticks were placed in Barbour-Stoenner-Kelly (BSK II) culture medium and incubated at 32°C for 4 wk. Cultures were examined by darkfield microscopy for the presence of spirochetes.

To determine whether immunized mice became infected after challenge by naturally infected ticks, selected tissues were excised and cultured or examined for pathognomonic lesions. Mice were killed 1 mo after the ticks had engorged. Blood, spleen, bladder, and skin were collected aseptically from each mouse, homogenized (spleen and bladders), and cultured in BSK II medium. Cultures were incubated for 2 wk and examined by darkfield microscopy. Joints (knee and tibiotarsal) and hearts were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Inflammation within the joints or carditis (13, 14) were considered as evidence of disease. All such slides were examined without knowledge of immunization status.

To determine whether spirochetes infecting ticks collected from the Nantucket Field Station site may be antigenically variable, aliquots of tick gut homogenates were stained by indirect immunofluorescence using a battery of mAbs. Briefly, intact guts were dissected from six adult *I. dammini* collected from the identical transects from which the challenge nymphs were collected, and individually homogenized in 500 μl of PBS (pH 7.2) with 0.5% BSA. After a 10-s low-speed centrifugation to pellet gross tissue debris and cuticle, aliquots of 5 μl were spotted onto wells of 12-well IFA slides (Cel-Line, Newfield, NJ), dried at room temperature, and fixed in cold acetone for 10 min. mAbs against OspA (H5332 and H3TS, courtesy of A. G. Barbour; and IXDII87, 8C4B5C, and VIIIC378 [3]), flagellin (H9721 and H604, A. G. Barbour), and OspB (H6831, A. G. Barbour) were applied as undiluted hybridoma supernatants. High-titered rabbit immune serum and normal rabbit serum were applied as comparisons. After incubation for 1 h, and brief washing in PBS, appropriate secondary antibodies (antirabbit IgG or polyvalent antimouse Ig) labeled with FITC were applied to the slides, and allowed to react for 30 min. The slides were washed in PBS, mounted in glycerol, coverslipped, and examined by epifluorescent microscopy at ×312. All spirochetes with 3+ fluorescence were counted within each 5-μl aliquot. Each antibody was applied to three replicate aliquots of homogenate from each tick. Counts derived for each mAb were pooled for all ticks with respect to antigen (e.g., OspA or flagellin), and examined by analysis of variance (ANOVA).

Results and Discussion

First, we determined whether spirochetes delivered by naturally infected nymphal ticks infect mice that had been immunized either with recombinant OspA or OspB immunogen. No spirochetes could be cultured from the tissues of any of these vaccinated mice (Table 1), and suggestive histopathological lesions could be detected only in one individual of each vaccinated group of mice. In contrast, spirochetes multiplied in cultures inoculated with ear skin taken from about a third of carrier-immunized mice (χ^2, $p < 0.01$), and pathognomonic lesions were noted in almost half (carditis, $p < 0.01$; arthritis, $p < 0.001$). We concluded that either Osp immunogen protects mice against infection by field-derived, tick-transmitted spirochetes.

Table 1. *Efficacy of Protection by Vaccination with Recombinant Spirochetal Osp's*

Immunogen	Challenged mice			
	No. mice examined	Percent culture positive	Percent with arthritis	Percent with carditis
OspA	24	0	4	0
OspB	17	0	0	6
GT	32	34	41	44

Tissues were taken from tick-challenged mice 1 mo after infection, and assayed for evidence of infection by culture or histopathology.

Because protection appears to derive in part from immune-mediated destruction of spirochetes within the gut of the vector (4), we determined whether engorged ticks retained spirochetal infection (Table 2). Spirochetes were evident in fewer ticks that had fed on Osp-vaccinated mice than in those that had fed on sham-vaccinated mice ($p < 0.001$ and < 0.05, respectively). Spirochetes native to Nantucket Island do not survive when exposed to immune factors induced by vaccination with either Osp antigen.

We demonstrated that spirochete populations within the guts of ticks collected from the Nantucket site are antigenically variable by examining their reactivity with various mAbs against OspA, OspB, and flagellin. In comparison with the number of spirochetes per aliquot of gut homogenate that reacted with a polyclonal rabbit immune serum, fewer

Table 2. *Elimination of Spirochetal Infection from the Guts of Ticks Engorging on Vaccinated Mice*

Immunogen	Challenge ticks	
	No. examined	Percent infected
OspA	34	3
OspB	19	11
GT	38	37

Ticks used to challenge vaccinated mice were examined for evidence of infection 10 d after feeding to repletion.

spirochetes from the identical homogenate reacted with the two groups of anti-OspA mAbs, or with those against OspB or flagellin (ANOVA, $f = 6.55$; $df = 4$; $p < 0.05$). Spirochetes with reactive OspB, in particular, appeared to be rare compared with those with reactive OspA (OspB mean, 17.9 per 5 μl vs. OspA mean, 124.3 per 5 μl; $p < 0.001$ by Student's t test). The possibility that tick gut–derived spirochetes were differentially reactive because of differences in titer or staining efficiency of the various mAbs that were used was excluded by a similar experiment using 5-μl aliquots of BSK-cultured B31 spirochetes, identical concentrations of reagents, and identical incubation times. Culture-derived spirochete counts did not differ between the various treatments with the mAbs (ANOVA, $f = 1.53$; $df = 4$; $p > 0.10$). We conclude that spirochetes within ticks collected from the Nantucket site are antigenically heterogeneous.

These observations suggest that although antigenic variability in products of the Osp operon (15, 16) may be frequent in nature, vaccine-induced immunity is not affected. Because the mouse reservoir hosts are repeatedly exposed to infection, with each mouse receiving as many as six to eight infectious bites each month (S. R. Telford III, unpublished observations), it may be that ticks acquire diverse spirochetal clones. The epidemiological significance of antigenic variability within the guts of ticks, however, remains speculative. Only a few spirochetes appear to be transmitted by infecting ticks (17, 18), perhaps between 100 and 1,000. The likelihood, therefore, that an vaccine escape variant will be transmitted by an infecting tick seems remote. Osp mutant spirochetes present within a zoonotic site thus may only rarely cause vaccine failure.

We were surprised to find that protection is conferred by the OspB immunogen as well as by that produced by OspA. Because OspB appears to be more variable than OspA (6), the efficacy of an OspB-based vaccine might be expected to be less than one based on OspA. On the other hand, it may be that naturally occurring OspB variants are poorly infections or less pathogenic than spirochetes containing the prototypic OspB-B31. Few spirochetes within the guts of the Nantucket ticks reacted with mAb H6831. Perhaps many ticks in nature contain poorly infectious, OspB variant spirochetes that are morphologically indistinguishable from those that are infectious. The effects of anti-OspB immunity may thus be more effective by destroying the few "infectious" spirochetes within the guts of ticks.

Vaccines containing the OspA or OspB epitopes may assist efforts designed to reduce risk of human Lyme disease. The agent of Lyme disease has been enzootic on Nantucket as long as it has elsewhere in the northeastern United States (19). Indeed, the Lyme disease epidemic seems to have begun there even before it emerged in coastal Connecticut in 1974. Nantucket may thus be representative of other coastal northeastern U.S. zoonotic sites, which account for as much as 90% of the Lyme disease cases reported in the U.S. each year (20). Even if the efficacy of Osp vaccination does not extend worldwide, local vaccine protection would constitute a noteworthy public health achievement.

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