OspB Antibody Prevents *Borrelia burgdorferi* Colonization of *Ixodes scapularis*

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Received 9 October 2003/Returned for modification 14 November 2003/Accepted 25 November 2003

*Borrelia burgdorferi* outer surface protein OspB is expressed by spirochetes in the *Ixodes scapularis* gut. OspB is transcribed from a bicistronic operon with *ospA*, a known spirochete adhesion gene in the tick gut. Here we examine whether OspB also has a specific function in ticks. OspB specifically binds to a protein or protein complex within the tick gut. We also assessed whether selected nonborreliacidal OspB antibodies or F(ab)2 fragments interfere with *B. burgdorferi*-tick attachment in vivo. We examined engorged ticks that fed on *B. burgdorferi* N40-infected *scid* mice that had been treated with OspB F(ab)2 fragments. Control F(ab)2 fragments did not interfere with *B. burgdorferi* colonization of the tick gut, whereas OspB F(ab)2 fragments significantly inhibited the attachment of spirochetes to the tick gut. These studies show that nonborreliacidal OspB antibodies interfere with *B. burgdorferi* colonization of *I. scapularis*, highlighting a specific role for OspB in spirochete-arthropod interactions and suggesting new antibody-mediated strategies for interfering with *B. burgdorferi* transmission.

*Borrelia burgdorferi* is responsible for Lyme disease, the most common vector-borne disease in the United States (16, 22). The bacterium is maintained in an enzootic life cycle, which primarily involves *Ixodes scapularis* ticks and mice (3). *Ixodes* ticks can feed on a wide range of vertebrate hosts, including humans, and deposit spirochetes into the skin during engorgement. The bacteria soon spread, often causing a skin rash known as erythema migrans in humans, and after several weeks may disseminate to distant organs, resulting in arthritis, carditis, and neurological disease (22). Laboratory mice infected with *B. burgdorferi* develop symptoms that are reminiscent of the human disease, including inflammation of the joints and heart. Inbred mice therefore serve as a reliable model for the study of experimental Lyme borreliosis (1).

*B. burgdorferi* is exquisitely adapted to survive in diverse host locations. Differential gene expression by the spirochete is thought to contribute to this adaptive process. For example, *B. burgdorferi* synthesizes BBK32 (6), a fibronectin-binding protein, and DbpA (11), a decorin-binding protein, early in mammalian infection, and these proteins are believed to be involved in spirochete pathogenicity. On the other hand, OspA, a lipoprotein, is primarily expressed by *B. burgdorferi* in ticks and generally is downregulated in mammals (20). Spirochetes swiftly upregulate OspA when entering ticks from an infected host and continue to produce abundant OspA within the resting tick (4). This preferential expression of *ospA* within *I. scapularis* suggests that OspA has a function within the vector. A recent study showing that OspA mediates spirochete adherence within the tick gut by binding to an *I. scapularis* protein supports this contention (18). Furthermore, nonborreliacidal OspA antibodies can inhibit *B. burgdorferi* attachment to the tick gut (19), highlighting the importance of OspA in spirochete-tick interactions in vivo and indicating how stage-specific gene expression contributes to the maintenance of the natural cycle of the spirochete.

The *ospA* and *ospB* genes are organized into a single operon under the control of a common promoter (12). Most studies have focused on OspA, and less information is available on the role of OspB during the life cycle of *B. burgdorferi*. Several reports, however, have indicated that OspB is present on the surface of *B. burgdorferi* within unfed ticks (2, 17, 23). Certain OspB antibodies, either as whole immunoglobulin G (IgG) or as Fab fragments, can be bactericidal in vitro, and studies have demonstrated that vaccination with OspB can protect mice from *B. burgdorferi* infection (5, 7, 13, 14). Because OspB is located on the surface of *B. burgdorferi*, expressed by spirochetes within ticks, and encoded on a bicistronic operon with *ospA*, we examined the role of OspB in *B. burgdorferi*-I. scapularis interactions.

**MATERIALS AND METHODS**

*B. burgdorferi* and I. scapularis. A low-passage-number clonal isolate of *B. burgdorferi* N40 that is infectious to mice was used throughout the study (18). Adult female *I. scapularis* ticks were collected in Connecticut. The egg mass was laid in the laboratory. Hatched larvae were allowed to feed on uninfected C3H mice to produce pathogen-free nymphs. All tick rearing was performed in an incubator at 26°C in 85% relative humidity with a 12-h light-dark cycle.

Enzyme-linked immunosorbent assay (ELISA) and confocal microscopy to assess protein binding to TGE. Recombinant OspB and ErpT (a representative control protein) from *B. burgdorferi* N40 were expressed and purified in their nonlipidated forms (18). OspB and ErpT were expressed either without a fusion partner or as fusion proteins with glutathione transferase (after which the fusion partner was cleaved by use of a protease), as previously described (18). OspB, ErpT, or bovine serum albumin (BSA) was labeled with fluorescein isothiocyanate (FITC) from Molecular Probes (Eugene, Oreg.). The extent of conjugation of FITC per molecule of protein was determined according to the manufacturer’s instructions. One microgram of each FITC-labeled protein represents 35 pmol of OspB, 31 pmol of ErpT, and 15 pmol of BSA. One picomole of OspB, ErpT, and BSA bound to 2.2:1 and 6.5 pmol of FITC, respectively. Guts from flat nymphal *I. scapularis* ticks were dissected in phosphate-buffered saline (PBS) and homogenized on ice with a Kontes microhomogenizer (VWR Scientific Products, West Chester, Pa.).
A aliquot of TGE was also incubated with trypsin for 1 h. Enzyme treatment was done with a glycoprotein deglycosylation kit (Sigma). An equal volume of 50 μl of TGE was preincubated with either heat-inactivated 5% calf serum for 30 min at room temperature, and then incubated for 1 h at room temperature with FITC-labeled OspB, ErpT, or BSA (50 μg/ml of TGE coated wells, ErpT, or BSA (50 μg/ml) at 37°C. The plates were washed three times with PBS-Tween 20. Binding was detected by using anti-FITC IgG horseradish peroxidase (Amersham Pharmacia Biotech, Piscataway, N.J.) as a secondary reagent, and TMB microwell peroxidase substrate (KPL, Gaithersburg, Md.) was used for color development. The optical density (OD) was read at 450 nm at 15 min.

The assessment of protein binding by confocal microscopy was performed as previously described (19). Five to 10 mid-guts were dissected from nymphal ticks allowed to feed to repletion and detach from the mice, which usually occurred at 72 to 96 h. Guts from each group of nymphs were dissected under a dissecting microscope equipped with an argon-krypton laser.

Treatment of the tick gut with lipase, glycosidase, or trypsin. TGE was prepared as described above, and equal aliquots were incubated with either heat-inactivated (95°C for 10 min) or active lipase or glycosidases as follows: wheat germ lipase was used at 10 U/ml (Sigma) for 1 h at 37°C, and O-glycosidase, PNGase F, α(3,6,8,9)-neuraminidase, β-N-acetylgalcosaminidase, and β(1-4)-galactosidase treatment was done with a glycoprotein deglycosylation kit (Sigma). An equal aliquot of TGE was also incubated with trypsin for 1 h at 37°C at 10 μg/ml (Sigma) in the presence or absence of 20 μg of soybean trypsin inhibitor (Sigma)/ml. After enzyme incubation, the TGE and enzyme-treated TGE were used to coat microtiter plates and were probed with labeled OspB as described for ELISAs.

Antibodies and generation and characterization of Fab(α)2 fragments. The generation of OspB polyclonal or monoclonal antibodies (MAbs) B22J (B22) and B27G (B27) against B. burgdorferi N40 has been described previously (7). Ten milliliters of normal rabbit sera or polyclonal antisera was passed over a 0.5 ml protein A column (Bio-Rad Laboratories) that was then washed twice with 20 ml of PBS, pH 7.4. The bound IgG was eluted in 1 ml of 0.1 M glycine, pH 3.0. The Ab was then concentrated and desalted in a spin column (Amicon, Beverly, Mass.), and the protein concentration was determined by use of a Bio-Rad protein assay kit (Bio-Rad Laboratories), with BSA (American Bioanalytical, Natick, Mass.) as the standard. We used immobilized pepsin to generate Fab(α)2 fragments from the whole IgG fraction of normal rabbit sera or OspB antisera (ImmunoPure Fab(α)2 preparation kit; Pierce, Rockford, Ill.). The cleaved Fab(α)2 fragments were then separated from Fe fragments or undigested IgG F(ab)2 fragments and were concentrated in a spin column according to the manufacturer’s instructions. The purity of the Fab(α)2 fragments was checked by running an aliquot through a sodium dodecyl sulfate-12% polyacrylamide gel. The binding of OspB Fab(α)2 to B. burgdorferi lysates and whole spirochetes was analyzed by a standard ELISA and immunofluorescence, respectively, as described previously (19).

Bactericidal assay. Antibodies or F(ab)2 fragments were tested for their bactericidal activity against B. burgdorferi N40 by dark-field microscopy as described previously (19). Briefly, spirochetes (5 × 10⁶/ml) were incubated in Barbour-Stoenner-Kelly (BSK) medium with 20% OspB polyclonal sera, control sera, or OspB-producing hybridoma culture supernatants as well as 50 μg of purified Fab(α)2 fragments/ml for 48 h at 33°C. The percentage of viable spirochetes was determined by dark-field microscopic observation of the loss of spirochete motility and retractility from 10 random fields in a double-blinded manner. In addition, 50-μl aliquots from each control or antibody-treated group were removed and incubated with 500 μl of BSK medium at 33°C for 5 days. The B. burgdorferi cells were then counted, and these results were compared with the initial viability by dark-field microscopy.

In vivo infection and adherence studies. Pathogen-free NCr immunodeficient mice (Ncr-SCID) from the National Institutes of Health (Bethesda, Md.) were infected with B. burgdorferi N40 (10⁶ spirochetes/mouse, three animals per group) by intradermal injection into the back. After 3 weeks, selected F(ab)2 fragments (100 μg/mouse) or MAbs (200 μg/mouse) were administered to groups of mice (100 μl intraperitoneally and 100 μl subcutaneously). Twenty-four hours later, 10 I. scapularis nymphs were placed on each mouse. Antibodies or Fab(α)2 fragments or antibodies on the next day. The nymphs were allowed to feed to repletion and detach from the mice, which usually occurred at 72 to 96 h. Guts from each group of nymphs were dissected under a microscope in PBS (20 ml/gut) and were examined 24, 48, and 72 h after tick detachment. Five-microliter aliquots were examined for viable spirochetes by dark-field microscopy.

From these experiments, the difference between the binding of OspB to TGE and that of either BSA (P < 0.001) or ErpT (P < 0.005) was significant.

OspB binds to the I. scapularis gut. We first assessed whether OspB bound to a TGE by using an ELISA-based assay. Wells were coated with TGE and probed with FITC-labeled recombinant OspB from B. burgdorferi N40, a prototypic infectious B. burgdorferi sensu stricto isolate. We detected significant binding of OspB to TGE in comparison to other control proteins, such as BSA or B. burgdorferi ErpT, which adhered poorly to the TGE (Fig. 1). OspB only bound weakly to antigens in the fetal bovine serum (Fig. 1). The capacity of OspB to adhere to the tick gut was further examined by confocal microscopy. FITC-labeled OspB bound to the intact unfixed tick gut (Fig. 2), while FITC-labeled ErpT or FITC-labeled BSA did not bind to the tick gut (Fig. 2).
OspB binding to the tick gut is abolished by trypsin treatment. The OspB receptor in ticks was next partially characterized. For an understanding of the biochemical nature of the OspB binding moiety, the TGE was treated with wheat germ lipase, a variety of glycosidases [O-glycosidase, PNGase F, α2(3,6,8,9)-neuraminidase, β-N-acetylgalcosaminidase, and β(1-4)-galactosidase], or trypsin. Heat-denatured enzyme (in the case of lipase or glycosidases) and protease in the presence of a specific inhibitor (soybean trypsin inhibitor) served as controls. Soybean trypsin inhibitor alone also served as a control and did not alter the binding of OspB to the TGE (not shown). While glycosidases or lipase treatment did not affect OspB binding to the TGE, pretreatment of the TGE with trypsin markedly diminished OspB adherence (Fig. 3). These data suggest that OspB binds to a protein or protein complex in the I. scapularis gut.

OspB F(ab)2 fragments lack in vitro bactericidal activity but inhibit the association of spirochetes with the tick gut in vivo.

Previous studies have indicated that B. burgdorferi OspA plays an important role in the attachment of spirochetes to the tick gut (19). Since, like OspA, OspB also binds to the tick gut, we were interested in determining whether B. burgdorferi N40 exposed to OspB antibody could effectively colonize I. scapularis. Certain OspB antibodies or F(ab)2 fragments have been shown to exert in vitro bactericidal activity, so we first characterized whether selected OspB antibodies or F(ab)2 fragments can kill the spirochete in vitro. We prepared F(ab)2 fragments from a polyclonal OspB antiserum and used MAb B22 or MAb B27 as whole IgG, as these MAbs were previously characterized as nonbactericidal and bind to defined N-terminal epitopes of OspB as well as to the surface of unfixed B. burgdorferi N40 (7). MAb B22 binds an OspB epitope within amino acids 34 to 91, and MAb B27 binds a region within amino acids 134 to 167 (7). Both MAbs only react with OspB in an immunoblot with B. burgdorferi lysates (7). As expected, neither MAb B22 or MAb B27 or OspB F(ab)2 fragments were found to exert significant bactericidal activity against B. burgdorferi N40 grown in vitro (Table 1). For in vivo adherence studies, SCID mice were used because the animals cannot mount their own humoral response against B. burgdorferi during infection. The effects of the administered antibodies or F(ab)2 fragments can therefore be evaluated without the contribution of any acquired host response to the spirochete. Groups of three SCID mice were challenged with B. burgdorferi N40, and within 3 weeks all the mice had developed visible swelling of the tibiotarsal joints due to disseminated infection with the spirochete. Next, either normal rabbit serum, F(ab)2 fragments prepared from a polyclonal OspB antiserum and used MAb B22 or MAb B27 as control (B). Bars represent the OD450 values at 15 min and are expressed relative to controls without antibody or serum treatment. Data represent the mean numbers of spirochetes remaining viable after treatment (means ± standard errors from three independent experiments). Differences between the control and normal rabbit serum as well as OspB antibodies or F(ab2)-treated samples were not statistically significant (Student’s t test).

Table 1. Bovricidal activity of OspB antibodies against B. burgdorferi N40

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No. of viable B. burgdorferi cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no addition)</td>
<td>100</td>
</tr>
<tr>
<td>NRS</td>
<td>100</td>
</tr>
<tr>
<td>NRS-F(ab)2</td>
<td>93 ± 10</td>
</tr>
<tr>
<td>OspB-F(ab)2</td>
<td>93 ± 10</td>
</tr>
<tr>
<td>MAb B22</td>
<td>92 ± 4.5</td>
</tr>
<tr>
<td>MAb B27</td>
<td>99 ± 19</td>
</tr>
<tr>
<td>Human B. burgdorferi antiserum</td>
<td>10 ± 9</td>
</tr>
<tr>
<td>* SPIROCHETES WERE INCUBATED IN BSK MEDIUM IN THE ABSENCE (CONTROL) OR PRESENCE OF NORMAL RABBIT SERUM (NRS), F(ab2) FRAGMENTS PREPARED FROM NORMAL RABBIT SERUM [NRS-F(ab)2], POLYCLONAL ANTI-OSPB SERUM [OSPB-F(ab)2], OSPB MAb B22 OR B27 OR A BORERISALAR SERUM FROM A PATIENT WITH LYME DISEASE. THE NUMBERS OF SPIROCHETES WERE ASSESSED BY DARK-FIELD MICROSCOPY AFTER 24 AND 48 h AND ARE EXPRESSED RELATIVE TO CONTROLS WITHOUT ANTIBODY OR SERUM TREATMENT. DATA REPRESENT THE MEAN NUMBERS OF SPIROCHETES REMAINING VAILABLE AFTER TREATMENT (MEANS ± STANDARD ERRORS FROM THREE INDEPENDENT EXPERIMENTS). DIFFERENCES BETWEEN THE CONTROL AND NORMAL RABBIT SERUM AS WELL AS OSPB ANTIBODIES OR F(ab2)-TREATED SAMPLES WERE NOT STATISTIICALLY SIGNIFICANT (STUDENT'S T TEST).</td>
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colonization of *B. burgdorferi* within *I. scapularis*. The distribution of *B. burgdorferi* within the *I. scapularis* gut 24 and 72 h after feeding is shown. Nymphal ticks fed on *B. burgdorferi*-infected mice that had been treated with either normal rabbit serum (NRS), F(ab)_2 fragments from normal rabbit serum (N-Fab), or OspB MAb B22 or B27 or F(ab)_2 fragments prepared from polyclonal anti-OspB sera (B-Fab). The spirochetes (arrows) were stained with a FITC-labeled goat anti-OspB antibody (green), and the nuclei of the gut epithelial cells were stained with propidium iodide (red). Images were recorded at ×400 magnification and are presented as a merged image for clarity.

**Table 2.** Effect of OspB antibodies on attachment of *B. burgdorferi* to the tick gut

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>NRS</th>
<th>NRS-Fab</th>
<th>MabB22</th>
<th>MabB27</th>
<th>OspB-Fab</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>121 ± 28</td>
<td>159 ± 57</td>
<td>265 ± 88</td>
<td>221 ± 142</td>
<td>7 ± 1.6</td>
</tr>
<tr>
<td>48</td>
<td>199 ± 45</td>
<td>102 ± 51</td>
<td>135 ± 37</td>
<td>47 ± 11</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>72</td>
<td>227 ± 34</td>
<td>117 ± 29</td>
<td>297 ± 74</td>
<td>205 ± 55</td>
<td>5 ± 2.3</td>
</tr>
</tbody>
</table>

OspB antibodies, either as whole IgGs or Fab fragments, have been reported to be either bactericidal or nonbactericidal in vitro (7, 13, 15). The lack of an in vitro bactericidal effect of the MAb B22 or B27 in our studies supports an earlier obser-

**FIG. 4.** OspB F(ab)_2 fragments interfered with the attachment and colonization of *B. burgdorferi* within *I. scapularis*. The distribution of *B. burgdorferi* within the *I. scapularis* gut 24 and 72 h after feeding is shown. The spirochetes (arrows) were stained with a FITC-labeled goat anti-OspB antibody (green), and the nuclei of the gut epithelial cells were stained with propidium iodide (red). Images were recorded at ×400 magnification and are presented as a merged image for clarity.

**DISCUSSION**

*B. burgdorferi* selectively produces distinct sets of proteins to enhance infectivity and to survive in mammalian or arthropod hosts (3). OspA and OspB are examples of spirochete proteins which are abundantly synthesized in ticks (10, 17, 20). *B. burg-
viation that such antibodies failed to provide protective immu-

nity in vivo (7). Interestingly, F(ab)2 fragments prepared from a polyclonal OspB antiserum also failed to show significant killing of B. burgdorferi in our in vitro assay. Although MAbs B22 and B27 as well as OspF F(ab)2 fragments were found to be nonborreliacidal, these antibodies can bind to the surface of B. burgdorferi N40 (7). We therefore used them for further in vivo adherence studies with the murine model of tick-transmitted Lyme borreliosis.

Our in vivo studies examined the capacity of nonbactericidal OspB F(ab)2 fragments or antibodies to prevent effective tick colonization by B. burgdorferi. We administered the F(ab), fragments or antibodies to B. burgdorferi-infected mice, allowed ticks to acquire the antibodies together with the spirochetes, and assessed whether gut tissue colonization by spirochetes is affected in the presence or absence of OspB antibodies. Our in vivo studies demonstrated that OspB F(ab)2 fragments, even those that did not kill spirochetes in vitro, effectively prevented B. burgdorferi from associating with the I. scapularis gut. Such OspB antibodies or F(ab)2 fragments may bind directly to the tick gut binding region of OspB. It is likely that the tick gut binding site of OspB is recognized by antibodies that do not bind the epitopes identified by either MAb B22 or B27. Alternatively, these polyclonal antibody fragments may bind to several epitopes of OspB on the B. burgdorferi surface, and steric hindrance might then interfere with OspB binding to the tick gut. Since OspA is also involved in the attachment of spirochetes to the tick gut and since we have found that polyclonal OspB antibodies prevented the binding of spirochetes, it is possible that steric hindrance by OspB antibodies also affected OspA-mediated binding of spirochetes to the tick gut. This is not surprising, as both lipoproteins have been shown to be colocalized on the spirochete surface (5). Overall, these results, together with our in vitro studies on OspB binding to the tick gut, indicate that OspB serves as a functional ligand for B. burgdorferi-tick gut interactions. The mechanism by which B. burgdorferi interacts with ticks and survives therein remains to be explored. The characterization of B. burgdorferi ligands that play significant roles in bacterial colonization and survival at the arthropod-pathogen interface will enhance our knowledge of vector-spirochete interactions and highlight the contribution of differentially expressed genes in the enzootic life cycle of B. burgdorferi.

ACKNOWLEDGMENTS

The human experimentation guidelines of Yale University were followed in the conduct of clinical research, and animal experimentation guidelines were followed for animal studies.

This work was supported by grants from the NIH. E. Fikrig is the recipient of a Burroughs Wellcome Clinical Scientist Award in Translational Research. R. A. Flavell is an investigator of the Howard Hughes Medical Institute.

We sincerely thank Debby Beck, Syed A. Morshed, and Denise Lusitani for their help in the study and Fran Manzo for help with manuscript preparation.

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Editor: D. L. Burns