Analysis of Antibody Response in Humans to the Type A OspC Loop 5 Domain and Assessment of the Potential Utility of the Loop 5 Epitope in Lyme Disease Vaccine Development

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The OspC protein of Borrelia burgdorferi is an immunodominant antigen. Here we demonstrate that the loop 5 domain of type A OspC is surface exposed, elicits bactericidal antibody in mice, and is antigenic in humans. The data suggest that loop 5 may be suitable for inclusion in a polyvalent, chimeric OspC vaccinogen.
strongest immunoreactivity with the loop 5 fragment and hence were selected for further analysis.

To more accurately define the residues within the loop 5 domain that are recognized by infection-induced antibody, PepSpot arrays were screened with the sera from patients 8 and 44 and with serum from mice infected with a clonal population of the type A OspC-producing strain B31MI (6). The PepSpot arrays consisted of 12- to 13-residue overlapping peptides (two-amino-acid step) spanning the loop 5 domain of type A OspC spotted onto Whatman 50 cellulose membrane (~150 nmol/cm²; JPT Peptide Technologies GmbH, Berlin, Germany). The PepSpot membranes were blocked (5% nonfat dry milk in Tris-buffered saline–0.5% Tween 20), washed, and screened with mouse and human serum samples (diluted 1:1,000 and 1:400 in blocking solution, respectively), and antibody binding was detected with species-specific anti-IgG antisera. Although the specific residues that make up the immunoreactive domain differed slightly in mice and humans, the major epitopes localized within residues 130 to 146 (Fig. 2). In type A OspC sequences, this region encompasses the C-terminal region of alpha helix 3 and the N-terminal portion of loop 5.

The crystal structures of OspC spatially place loop 5 on a prominent bend of the protein (7, 17). This loop has been postulated to be part of a potential ligand-binding pocket (17). To determine whether loop 5 is displayed at the cell surface and is accessible to antibody in in vitro grown spirochetes, immunofluorescence assays (IFAs) were performed by using anti-loop 5 antiserum. Immunoblot analyses with whole-cell lysates of B. burgdorferi B31 MI (type A OspC), B. parkeri, and S-tagged r-type A loop 5 demonstrated that the loop 5 antigen is specific, establishing the suitability of this antiserum for IFAs (Fig. 3A). The strains analyzed by IFA consisted of B. burgdorferi B31MI (type A OspC) and LDP74 (type K OspC). The spirochetes were grown at 33°C and transferred to 37°C for 3 days to stimulate OspC expression. IFAs were conducted with permeabilized cells (acetone fixed), nonpermeabilized cells (air dried), and standard methods as previously described (24). The slides were screened with a 1:1,000 dilution of mouse α-loop 5 antiserum, mouse preimmune serum, or rabbit-α-flagellin antiserum. Detection was achieved by using Alexa Fluor 568-conjugated goat α-mouse IgG or Alexa Fluor 488-conjugated goat α-rabbit IgG (10 μg/ml in blocking buffer). Slides were visualized on an Olympus BX51 fluorescence scope using a rhodamine or fluorescein filter set, as appropriate, or by dark-field microscopy, and photographed by using an Olympus MagnaFIRE camera. The labeling observed by IFA was highly specific and consistent with the immunoblot analyses; the type A-producing isolate was surface labeled (Fig. 3B), while the B. burgdorferi LDP74 type K OspC was not (Fig. 3C). In addition, consistent with the upregulation of OspC at elevated temperature, IFAs revealed markedly greater surface labeling of spirochetes grown at 37°C than cells grown at 33°C. The α-FlaB antiserum, which recognizes an inner-membrane-anchored, periplasmic protein, did not label nonpermeabilized cells but readily labeled cells permeabilized with acetone (data not shown). This control demonstrates that the loop 5 epitope is in fact surface exposed and that the experimental conditions used in the IFA did not disrupt cell integrity and thereby
artificially expose epitopes that are not naturally presented on the surface of the bacteria.

The ability of the loop 5 antiserum to efficiently bind to OspC at the cell surface raised the possibility that the interaction could be bactericidal, as has been demonstrated for antibody to full-length OspC (3, 14, 15, 19, 25). To determine whether antibody targeting loop 5 also exhibits bactericidal activity, killing assays were conducted with B. burgdorferi isolates B31MI and LDP74 cultivated at 33°C or temperature shifted to 37°C. The spirochetes were harvested by centrifugation, washed, and adjusted to 5 × 10^7 cells per 500 μl (in BSK-H medium), and 12.5 μl was transferred into a sterile 0.65-ml microcentrifuge tube. Then, 10 μl of heat-inactivated (56°C; 30 min) loop 5 serum was added with or without guinea pig complement (7.5 μl; Sigma Chemical, St. Louis, Mo.), the components were mixed and incubated at 33 or 37°C for 8 h. A total of 70 μl of H2O was added, and spirochetes were stained with the Live/Dead BacLight stain (Molecular Probes, Eugene, Oreg.) according to the manufacturer’s instructions. In brief, two stains are added to the cells; SYTO 9 and propidium iodide. These dyes can distinguish live bacteria (i.e., with intact membranes) from bacteria with compromised membranes. Live bacteria fluoresce green due to staining with SYTO 9, whereas dead or damaged bacteria fluoresce red due to staining with propidium iodide. The baseline level of cells with disrupted membranes observed upon treatment with preimmune heat inactivated serum (with or without complement) was ~25% (Fig. 3D). In contrast, ~70% of the cells exposed to the α-loop 5 antiserum displayed membrane disruption (Fig. 3E). The bactericidal activity was determined to be complement dependent. The blebbing effect seen here upon treatment with anti-loop 5 antibody is consistent with that reported with other anti-OspC antibodies (3, 8). It is also important to note that, consistent with the upregulation of OspC at elevated temperature, the percentage of dead cells was consistently higher in spirochetes grown at 37°C than in bacteria grown at 33°C (data not shown). It is clear from the data presented that anti-loop 5 antibody is bactericidal.

Several reports have outlined the clear and strong justification for the development of Lyme disease vaccines (reviewed in reference 12). However, at the present time, no vaccine is commercially available. In an effort to develop a broadly protective Lyme disease vaccine, Baxter pursued a strategy of generating a vaccine cocktail of 14 different full-length r-OspC proteins (12). However, the cocktail was deemed unacceptably reactigenic. The reactigenicity may have resulted from the large amount of protein that was required to elicit a sufficient response to the unique protective epitopes of each OspC type protein in the cocktail. A potential problem with cocktail vac-

FIG. 3. Demonstration that loop 5 is surface exposed and that antibody to loop 5 is bactericidal. The IFAs and bactericidal assays were conducted with antiserum generated against type A loop 5. (A) The results demonstrate the specificity of the anti-loop 5 antiserum. Whole-cell lysates of B. burgdorferi B31 MI, B. parkeri, and r-type A loop 5 fragment were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, immunoblotted, and screened with anti-type A loop 5 antiserum (1:1,000). Molecular masses of the protein markers are to the right of the figure. To assess the surface exposure of loop 5 on intact cells, IFAs were performed. (B and C) B. burgdorferi strains B31MI (type A [B]) and LDP74 (type K [C]) were grown at 37°C, washed with PBS, and fixed to slides by air drying. The slides were blocked and probed with a 1:1,000 dilution of mouse α-loop 5 antiserum or mouse preimmune serum as indicated. Secondary detection was by Alexa Fluor 568-conjugated goat α-mouse IgG (Molecular Probes) at 10 μg ml^-1. Magnification, ×400. Dark-field images for the mouse preimmune serum and the anti-type A loop 5 antiserum are shown in panels B and C. The loop 5 antiserum specifically labels only the type A cells. (D and E) To assess the potential bactericidal activity of the anti-loop 5 antiserum, bactericidal assays were performed. B. burgdorferi B31MI (type A) was incubated with preimmune sera (D) or mouse α-loop 5 antiserum in the presence of guinea pig complement (E). Staining was then performed by using the BacLight LIVE/DEAD assay system (Molecular Probes). Live cells stain green, whereas dead or damaged cells stain red. All procedures were performed as described in the text.
cines that use multiple full-length proteins is the potential for
direction of the antibody response to conserved, irrelevant,
nonprotective epitopes. It may be possible to overcome this
problem through the development of a chimeric, r-vaccigenen
composed of the naturally presented immunodominant linear
epitopes of each of the dominant OspC types. This general
concept has its origins in efforts to develop malaria vaccines
using epitopes from proteins expressed at different stages of
infection (12). The same concept has been applied in the de-
velopment of a hexavalent M protein vaccine for group A
streptococci (5) and in the development of vaccines against
several other pathogens with excellent success (2, 4, 9, 13, 16,
21, 31). With new insights into the physical and antigenic
structure of OspC, it may now be possible to develop an ef-
fective, r-polyvalent, chimeric, OspC vaccine. The newly iden-
tified loop 5 domain appears to be ideally suited for inclusion
in such a vaccine.

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