C-Terminal Invariable Domain of VlsE May Not Serve as Target for Protective Immune Response against *Borrelia burgdorferi*

FANG TING LIANG, MARY B. JACOBS, AND MARIO T. PHILIPP*

Department of Parasitology, Tulane Regional Primate Research Center, Tulane University Health Sciences Center, Covington, Louisiana 70433

Received 1 August 2000/Returned for modification 12 October 2000/Accepted 22 November 2000

VlsE, the variable surface antigen of the Lyme disease spirochete, *Borrelia burgdorferi*, contains two invariable domains, at the amino and carboxyl termini, respectively, which collectively account for approximately one-half of the entire molecule's length and remain unchanged during antigenic variation. It is not known if these two invariable domains are exposed at the surface of either the antigen or the spirochete. If they are exposed at the spirochete's surface, they may elicit a protective immune response against *B. burgdorferi* and serve as vaccine candidates. In this study, a 51-mer synthetic peptide that reproduced the entire sequence of the C-terminal invariable domain of VlsE was conjugated to the carrier keyhole limpet hemocyanin and used to immunize mice. Generated mouse antibody was able to immunoprecipitate native VlsE extracted from cultured *B. burgdorferi* B31 spirochetes, indicating that the C-terminal invariable domain was exposed at the antigen's surface. However, this domain was inaccessible to antibody binding at the surface of cultured intact spirochetes, as demonstrated by both an immunofluorescence experiment and an in vitro killing assay. Mouse antibody to the C-terminal invariable domain was not able to confer protection against *B. burgdorferi* infection, indicating that this domain was unlikely exposed at the spirochete's surface in vivo. We concluded that the C-terminal invariable domain was exposed at the antigen's surface but not at the surface of either cultured or in vivo spirochetes and thus cannot elicit protection against *B. burgdorferi* infection.

Like other vector-borne pathogens, such as the protozoan *Trypanosoma brucei* (9, 35), the spirochete *Borrelia hermsii* (5, 33) and the ehrlichia *Anaplasma marginale* (28), the Lyme disease spirochete, *Borrelia burgdorferi*, expresses a surface antigen that undergoes antigenic variation (36). It is well accepted that antigenic variation plays an essential role in the pathogenesis of African trypanosomiasis, relapsing fever, and cyclic rickettsemia, as immune evasion permits the establishment of chronic infection and disease (5, 9, 28, 33, 35). Initial data indicate that antigenic variation in *B. burgdorferi* also may be a factor in the pathogenesis of Lyme disease (36).

All known variable antigens consist of both variable and conserved portions. In antigens such as the variant surface glycoprotein (VSG) of *T. brucei* (9, 35) and the variable major protein (Vmp) of *B. hermsii* (5, 33), variable portions encompass almost 70% of the primary sequences. In contrast, in VlsE, the variable surface antigen of *B. burgdorferi*, it is the invariable portions that account for more than 70% of the entire length of this antigen's primary sequence (19, 36).

VlsE has a predicted molecular mass of 34 kDa in the B31 strain of *B. burgdorferi sensu stricto* (36). Two invariable domains, one at the amino terminus (96 amino acids) and the other at the carboxyl terminus (51 amino acids), encompass together approximately one-half of this molecule's length (36) (Fig. 1). The remainder is composed of a central variable domain that contains six variable regions (VRs) and six invari-

able regions (IRs). These two types of regions are interspersed with each other, and each constitutes about one-half of the variable domain's length (19, 36). IRs and invariable domains may provide unique targets for the development of a vaccine against *B. burgdorferi* infection. Our previous studies on five of the six IRs indicate that although some of them are exposed at the surface of the molecule, none are exposed on the surface of spirochetes cultured in vitro (19, 22). Thus, unless the molecular architecture of VlsE is different in vivo, IRs may not elicit a protective immune response against *B. burgdorferi* infection.

In the present study we examined if the C-terminal invariable domain is exposed, both at the surface of the VlsE molecule and of the spirochete, and if this domain could elicit a protective immune response in mice. To address these issues, we generated antibodies to the C-terminal domain by immunizing mice with a 51-mer synthetic peptide (Ct) conjugated to the carrier keyhole limpet hemocyanin (KLH). This peptide reproduced the sequence of the C-terminal invariable domain of VlsE from the B31 strain of *B. burgdorferi* (36). Mouse antiserum was used in an immunoprecipitation experiment of native VlsE to determine exposure of the C-terminal domain at the surface of the VlsE antigen. Cell surface exposure of this domain was assessed by both an indirect immunofluorescence experiment and an in vitro killing assay. Finally, mice immunized with the Ct-KLH conjugate were challenged with *B. burgdorferi* by tick inoculation to determine if the C-terminal domain could elicit protection.

* Corresponding author. Mailing address: Tulane Regional Primate Research Center, Tulane University Health Sciences Center, 18703 Three Rivers Rd., Covington, LA 70433. Phone: (504) 871-6221. Fax: (504) 871-6390. E-mail: philipp@tpc.tulane.edu.

MATERIALS AND METHODS

Spirochetes. *B. burgdorferi sensu stricto* strain B31 spirochetes were cultivated in BSK-H medium supplemented with 10% rabbit serum (Sigma Chemical Co.,

FIG. 1. Diagrammatic illustration of the VlsE structure and the sequence of the C-terminal invariable domain. VlsE contains one amino- and one carboxyl-terminal invariable domain and one central variable domain. The variable domain consists of six VRs and six IRs. The amino acid sequence presented at the bottom of the figure was derived from the C-terminal invariable domain of VlsE from *B. burgdorferi* sensu stricto clonal isolate B31-5A3 (36).

St. Louis, Mo.) as described previously (29). Spirochetes grown to either mid-logarithmic phase or stationary phase were used in this study.

Peptide synthesis and conjugation to biotin and KLH. Two peptides were prepared using the fluorenylmethoxycarbonyl synthesis protocol (4). The Ct peptide reproduced the sequence of the C-terminal invariable domain of VlsE from *B. burgdorferi* isolate B31-5A3 (36), as shown in Fig. 1. The peptide C₆ was synthesized based on the IR₆ (subscript number indicates specific IR) sequence of VlsE from *Borrelia garinii* strain IP90 (19). A cysteine residue was included at the N terminus of each synthetic peptide and used as the conjugation site. Conjugation to biotin or KLH was performed by the *N*-succinimidyl maleimide carboxylate method. The maleimide reagent was from Molecular Probes (Eugene, Oreg.), and the protocol suggested by the manufacturer was followed. KLH was purchased from Pierce Chemical Company (Rockford, Ill.).

Mouse immunization and antiserum preparation. Four mice (6- to 8-week-old C3H/HeN; Charles River Laboratories, Wilmington, Mass.) were given three intraperitoneal injections at 3-week intervals of 100 µg of Ct-KLH conjugate emulsified with the MPL + TDM (M6536; Sigma). As controls, an additional four mice received three doses each of 100 µg of KLH alone emulsified with the same adjuvant. Two weeks after the last injection, the titer of antibody to the Ct peptide was assessed by a peptide-based enzyme-linked immunosorbent assay (ELISA), and the reactivity with the VlsE antigen was determined by immunoblotting using cultured-B31 spirochete lysates as antigen. Three weeks after the last boost, all of the animals were challenged by tick inoculation as described below.

Peptide-based ELISA. ELISA was performed as previously described (19).

Immunoblotting. B31 spirochetes grown to stationary phase were harvested and washed twice with phosphate-buffered saline (PBS) by centrifugation at $4,000 \times g$ for 20 min at 4°C. Washed spirochetes were suspended in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (125 mM Tris, 3% SDS, 5% β-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue, pH 6.8) at a concentration of 4×10^9 organisms per ml. The suspension was incubated at 95°C for 5 min. Approximately 130 µl of such preparation was applied to the sample lane of a two-well minigel of 12% polyacrylamide. Resolved proteins were transferred onto nitrocellulose in Towbin transfer buffer (34). The blot was shaken in blocking solution for 2 h and then placed in a Miniblotter 45 (Immunetics Inc., Cambridge, Mass.). Mouse serum samples that were collected at 0 and 8 weeks after immunization with Ct-KLH conjugate were diluted 1:200 with blocking solution and applied to the Miniblotter. The latter was then rocked for an additional 2 h. After three washes with PBS-Tween (PBS-T), the blot was incubated in 0.5 µg per ml of rabbit anti-mouse immunoglobulin G (IgG)-horseradish peroxidase conjugate for 1 h. After three washes with PBS-T, the blot was developed in PBS-T supplemented with 0.05% 4-chloro-1-naphthol, 0.015% H₂O₂, and 17% methanol.

Immunoprecipitation and immunoblotting. Immunoprecipitation was conducted as previously described (19). Dissolved precipitates were separated and electrotransferred to nitrocellulose membrane. The blot was probed with mouse anti-Ct serum that was collected at 8 weeks after immunization with Ct-KLH conjugate.

Indirect immunofluorescence. Immunofluorescence was performed as described previously (22). Both unfixed and fixed B31 spirochetes were incubated with mouse preimmune or anti-Ct serum. *B. burgdorferi*-infected mouse serum and anti-OspA (outer surface protein A) monoclonal antibody H5332 (purchased from the Department of Periodontics, University of Texas Health Sciences Center, San Antonio) were used as positive controls.

In vitro killing assay. B31 spirochetes were cultured in BSK-H medium until they reached mid-logarithmic phase (about 2×10^7 cells per ml). A total of approximately 5×10^5 spirochetes in 25 µl of BSK-H medium were added to

individual wells of a 96-well plate. A volume of 50 µl of heat-inactivated (56°C for 30 min) serum diluted 1:10 with the same medium was already dispensed in each well. The plate was incubated at 34°C for 30 min before the addition of 25 µl of complement (normal monkey serum) (27). After 24 h of incubation at 34°C in a humidified atmosphere of 3% CO₂, 5% O₂, and the balance of N₂, 5 µl was removed from each sample, and dead (nonmotile) and live (motile) spirochetes were counted under a dark-field microscope. A mouse MAb to OspA was used as the positive control (monoclonal antibody H5332).

Tick infection. Larval *Ixodes scapularis* kindly provided by Thomas Mather (University of Rhode Island, Kingston) were fed to repletion on mice that had been infected with *B. burgdorferi* B31 by needle inoculation. Larvae were allowed to molt in a humidified chamber at 22°C.

Mouse challenge infection and ear punch biopsy. Five nymphs were placed on the head and neck of each mouse and allowed to feed to repletion. Seventy percent of the nymphs were infected with B31 spirochetes as assessed by direct immunofluorescence with a fluorescein-labeled anti-*B. burgdorferi* antibody (Kirkegaard & Perry Laboratories, Gaithersburg, Md.). Ear punch biopsy specimens were obtained weekly for 4 weeks after tick inoculation and cultured at 34°C in BSK-H medium supplemented with 10% heat-inactivated rabbit serum. Cultured fluid was monitored weekly for the presence of spirochetes under a dark-field microscope for 1 month. To monitor infection, blood samples were also collected from all mice at 4 and 8 weeks after challenge and tested for antibody responses to both the C-terminal invariable domain and IR₆ by peptide-based ELISAs and to whole *B. burgdorferi* antigens by immunoblot.

RESULTS

Reactivity of mouse anti-Ct antibody with the VlsE protein.

To generate antibody to the C-terminal invariable domain of VlsE, the synthetic Ct peptide was covalently linked to KLH (Ct-KLH) and used to immunize mice. After three injections, the antibody titer in serum was determined by the peptide-based ELISA. The titer exceeded 1:25,600 in all of the mice that were immunized with Ct-KLH, whereas no anti-Ct antibody was detected in the mice given KLH alone (data not shown).

To assess if anti-Ct antibody recognizes the original protein, anti-Ct serum from each of the Ct-KLH-immunized mice was reacted with the VlsE antigen on immunoblots of a lysate of cultured B31 spirochetes. All of the immunized mice contained anti-VlsE antibody as assessed 2 weeks after the last injection (8 weeks after the initial infection) (Fig. 2). These results suggested that the synthetic peptide had retained, at least in part, the native conformation of the C-terminal invariable domain.

The C-terminal invariable domain is exposed at the surface of VlsE. To ascertain whether the C-terminal domain is exposed at the surface of the molecule, an immunoprecipitation experiment was performed using native VlsE protein. The latter was extracted from cultured spirochetes under mild conditions. Mouse anti-Ct antibody but not preimmune serum was

FIG. 2. Reactivity of mouse anti-Ct antibody with the VlsE antigen. A whole-cell lysate of cultured B31 spirochetes was separated by SDS-PAGE and transferred onto nitrocellulose. The blot was set up in a Miniblotter and reacted with sera collected from four mice (QC51, QC55, QC56, and QC58) at 0 and 8 weeks (WK) after the initial immunization with the Ct-KLH conjugate. Molecular masses are depicted at the left of the figure.

able to immunoprecipitate VlsE, indicating that the C-terminal domain was exposed at the surface of this antigen (Fig. 3).

The C-terminal domain is inaccessible to antibody at the surface of intact spirochetes. To assess if this domain is exposed at the spirochete's surface, an immunofluorescence experiment and an in vitro killing assay were conducted. Mouse anti-Ct antibody failed to label unfixed spirochetes, suggesting that the C-terminal invariable domain may not be exposed at the cell's surface (Fig. 4). Loss of the lp28-1 plasmid, which contains the *vlsE* locus (36), was ruled out as an alternative explanation of this result by the positive immunofluorescence result obtained with fixed spirochetes. Acetone fixation might remove or alter the distribution of membrane components that normally interact with the C-terminal invariable domain and thus artificially expose this domain to antibody. The inability of anti-Ct antibody to bind to live spirochetes contrasted with the bright fluorescence obtained with anti-*B. burgdorferi* antiserum. Anti-OspA antibody also labeled spirochetes (data not shown). The fact that intact spirochetes were able to bind anti-*B. burgdorferi* antibody but not anti-Ct antibody strongly indicates that the C-terminal invariable domain is not accessible to antibody binding at the spirochete's surface, at least in vitro.

The immunofluorescence results were consistent with that of the in vitro killing assay. As with mouse preimmune and anti-KLH serum, no significant killing was observed with anti-Ct-KLH antibody. In contrast, the anti-OspA monoclonal antibody yielded nearly 100% killing (Fig. 5).

Antibody to the C-terminal invariable domain fails to protect mice against *B. burgdorferi* infection. To examine if the C-terminal invariable domain is exposed at the in vivo spirochete's surface, mice immunized with either Ct-KLH or KLH were challenged with *B. burgdorferi* by tick inoculation. All of the four mice (QC44, QC45, QC46, and QC47) that received KLH and three of the four animals (QC51, QC55, QC56, and

QC58) that were given Ct-KLH became ear punch biopsy positive at 1 week postinoculation. Mouse QC51 remained negative during the entire biopsy specimen collection period (4 weeks). Thus, antibody to the C-terminal invariable domain was not able to significantly protect mice against challenge compared with the control group ($P > 0.5$; Fisher's exact test). This result is most likely due to the inaccessibility of this invariable domain to antibody binding in vivo. Alternatively, lack of VlsE expression in vivo also may explain the negative protection results. To monitor VlsE expression indirectly, antibody responses to both the C-terminal invariable domain and IR₆ were analyzed by peptide-based ELISAs. A strong antibody response to IR₆ was detected in both KLH- and Ct-KLH-immunized mice at 4 and 8 weeks postinoculation, indicating that VlsE was expressed during infection (Fig. 6). No anti-IR₆ antibody response was noted in mouse QC51 (Fig. 6). This result is consistent with the failure to culture spirochetes from biopsies collected from this mouse and confirms that it was not infected. The antibody response to *B. burgdorferi* was further analyzed by immunoblotting with whole-cell lysates of cultured spirochetes as antigen; multiple specific bands were detected with sera from all of the mice except QC51 (data not shown). However, ticks that were fed on this mouse to administer the challenge infection did contain spirochetes.

DISCUSSION

VlsE is an immunodominant surface antigen of *B. burgdorferi* (19–21, 23, 36). Its C-terminal invariable domain accounts

FIG. 3. Exposure of the C-terminal invariable domain at the surface of the VlsE antigen. VlsE extracted from cultured B31 spirochetes with solubilization buffer was immunoprecipitated with pooled mouse anti-Ct or preimmune serum in the presence of protein G-agarose beads. A spirochetal lysate and solubilized immunoprecipitates were separated by SDS-PAGE and blotted onto nitrocellulose. VlsE was visualized with the pooled mouse antiserum and goat anti-mouse IgG-peroxidase conjugate. In addition to VlsE (approximately 37 kDa), precipitated mouse IgG is visible at the top of the two lanes that contains the solubilized immunoprecipitates. Molecular masses are depicted at the left of the figure.

FIG. 4. The C-terminal invariable domain is inaccessible to antibody at the surface of intact spirochetes. Cultured *B. burgdorferi* B31 spirochetes were either fixed with acetone or left unfixed. Spirochetes were resuspended in PBS-bovine serum albumin containing mouse preimmune, anti-Ct, or anti-*B. burgdorferi* serum. Sensitized spirochetes were probed with goat anti-mouse IgG-fluorescein conjugate.

for 15% of the entire molecule and remains unchanged during antigenic variation (36–38). Moreover, because VlsE is a lipoprotein (36), its C terminus may be distal to the molecule's insertion point in the spirochetal membrane, and thus, in theory, it is the VlsE domain most likely to be exposed at the spirochetal surface. These premises provided a rationale to hypothesize that the C-terminal domain could serve as the target for a subunit vaccine. To examine this hypothesis, the 51-mer peptide Ct, which reproduced the entire sequence of the C-terminal invariable domain of VlsE, was synthesized, conjugated to KLH, and used to immunize mice. Mouse antibody was first assessed for reactivity with the synthetic peptide by using the Ct ELISA. High titers of antibody to this peptide in all of the four mice that were immunized with Ct-KLH were observed. Hence, the Ct peptide was immunogenic when administered in this fashion. This result did not automatically entail that the anti-Ct antibody would react with VlsE, for although the primary sequence of a polypeptide plays an essential role in determining its secondary and tertiary structures, other factors such as molecular chaperones are also crucial in the *in vivo* protein folding process (13). Antibody reactivity with the spirochetal VlsE antigen was examined using immunoblotting. The antipeptide antibody reacted with the VlsE molecule (Fig. 2). The observed reactivity suggested that the peptide retained the right conformation of the C-terminal invariable domain. This conclusion was further supported by both the results of the immunoprecipitation experiment, as the antipeptide antibody was able to precipitate the native VlsE (Fig. 3), and the immunofluorescence study, as the antibody

successfully labeled fixed spirochetes (Fig. 4). It also was consistent with the result of our antigenic analysis of the C-terminal invariable domain since the synthetic peptide reacted with antibody that had been generated during a *B. burgdorferi* infection by tick inoculation (Fig. 6). The observed molecular mass of the VlsE antigen was approximately 37 kDa (Fig. 2 and 3). This was higher than the predicted 34 kDa (36), probably because of the lipid moiety.

The molecular surface exposure of the C-terminal invariable domain was revealed by the immunoprecipitation experiment using native VlsE. Under this condition, only surface-exposed epitopes are likely able to bind to specific antibody. This conclusion was consistent with the result of the antigenic analysis of this domain. The C-terminal domain was immunodominant during *B. burgdorferi* infection in mice (Fig. 6). The antigenicity of this domain has also been noted in monkeys that have been infected with the spirochete (F. T. Liang and M. T. Philipp, unpublished data). Features of protein domains such as surface accessibility, hydrophilicity, flexibility, and proximity to a site recognized by helper T cells are all important in positively determining domain antigenicity (8). Unlike T-cell epitopes, all B-cell epitopes are presumably exposed at the antigen's surface (7).

Molecularly surface-exposed sequences of a surface protein may not be exposed at the bacterium's surface after the protein is inserted into the spirochetal outer membrane. To address this issue, two different experimental procedures were conducted: an immunofluorescence experiment and an *in vitro* killing assay. The result of the immunofluorescence experi-

FIG. 5. Lack of killing activity of anti-Ct antibody. Spirochetes were incubated with heat-inactivated mouse preimmune, anti-KLH, or anti-Ct-KLH serum or anti-OspA monoclonal antibody in BSK-H medium and monkey complement for 24 h. Dead and live spirochetes were counted under a dark-field microscope. Percent killing was calculated as $[\text{number dead}/(\text{number dead} + \text{number live})] \times 100$. Means of three determinations and standard deviations (error bars) are presented.

ment indicated that the C-terminal invariable domain was inaccessible to antibody at the surface of intact (unfixed) spirochetes (Fig. 4). This was further substantiated by the result of the in vitro killing assay, as absence of killing was most likely due to failure of the antibodies to bind to the spirochete's surface. The bactericidal activity of antibody to surface-exposed epitopes of *B. burgdorferi* through either complement-dependent or -independent mechanisms has been extensively reported (12, 24, 27, 32). Complement-mediated killing of *B. burgdorferi* is facilitated by antisurface antibody regardless of the complement-activating properties of the antibody. *B. burgdorferi* spirochetes are able to activate complement through an antibody-independent mechanism (16–18). Although the synthetic peptide essentially maintained the native conformation of the C-terminal invariable domain and mouse antibodies generated by immunization with this peptide were able to recognize the native VlsE antigen, the antibodies failed to either label or kill the spirochetes in vitro. These results strongly indicate that the C-terminal invariable domain is cryptic (tightly “packed”?) on the spirochetal surface, at least with spirochetes maintained in vitro. However, it is possible that the display of this domain is different in vivo.

To examine if the C-terminal invariable domain is exposed at the spirochetal surface in vivo, an immunization experiment was performed. Although mice produced high antibody levels to this domain by immunization with Ct-KLH conjugate, they were not significantly protected against challenge with *B. burgdorferi* by tick inoculation. This result suggests that the C-terminal invariable domain is inaccessible to antibody at the surface of the spirochete in vivo. Several experiments indicate that antibodies to surface antigens of *B. burgdorferi* can effectively protect mammalian hosts against infection (6, 14, 15).

It should be borne in mind, however, that lack of VlsE

FIG. 6. Mouse antibody responses to the C-terminal invariable domain and IR₆ after tick inoculation. Mice were immunized either with KLH (mice QC44, QC45, QC46, and QC47) or with Ct-KLH conjugate (mice QC51, QC55, QC56, and QC58) and then challenged with *B. burgdorferi* by tick inoculation. Serum samples were collected at 0 weeks (Pre) and 8 weeks (Post) after challenge and tested for the antibody response to both the C-terminal invariable domain and IR₆ by peptide-based ELISAs. The cutoff line (optical density [OD] = 0.27) was based on the mean + three standard deviations of the eight bleeds collected prior to immunization reacted individually with Ct and C₆.

expression also may allow the spirochete to survive in the presence of antibody to the C-terminal invariable domain even if this domain were exposed at the cell's surface. *B. burgdorferi* is able to change its protein expression profile as it is transmitted from the tick to the mammalian host, as well as in vitro (2, 26, 30, 31), and indirect evidence indicates that the expression of some spirochetal proteins varies during the course of infection in humans (1, 26). We indirectly monitored the expression of VlsE in vivo by analyzing the antibody response to IR₆ (Fig. 6), an immunodominant IR of VlsE (19–21, 23). Based on the early and persistent antibody response to IR₆, we conclude that VlsE is expressed during infection. This argues against the possibility that spirochetes not expressing VlsE were selected as survivors under the immunologic pressure of antibody to the C-terminal invariable domain. However, we did not assess whether spirochetes cultured from ear punch biopsy specimens of immunized mice expressed VlsE. In addition, the strong antibody response to the C-terminal invariable domain observed in all the four KLH-immunized mice indicated that this domain was immunodominant during *B. burgdorferi* infection in mice (Fig. 6). The antibody response to the C-terminal domain in the Ct-KLH-immunized mice cannot be simply explained by the immunogenicity of this domain since the specific antibody had already existed before challenge (Fig. 6).

VlsE, with a predicted molecular mass of 34 kDa, contains two invariable domains and one variable domain that consists of six VRs and six IRs (19, 36). Indirect evidence indicates that this antigen is surface exposed both in vitro (36) and in vivo (M. B. Lawrenz, J. M. Hardham, R. T.

Owens, and S. J. Norris, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. D/B-264, p. 260, 1999). The antigenicity and surface locations, both at molecular and cellular levels, of five IRs and one invariable domain have been investigated in the previous and present studies (19–23). Three of the IRs—IR₂, IR₄ and IR₆—are antigenic, and both IR₂ and IR₆ are immunodominant in mice and dogs (19, 20, 23). IR₆ appears to be universally antigenic since it is also immunodominant in humans and nonhuman primates (19–21). The C-terminal invariable domain is also immunodominant, at least in mice as shown in the present study. In contrast, the conserved portions of other variable antigens including VSG and Vmp have never been found to be antigenic in a natural infection, although they may induce an antibody response by immunization in the presence of adjuvant (3, 5, 11). Their variable domains, in contrast, are highly immunogenic and serve as the major target of host protective antibodies (9, 10, 33). The microorganisms express new variable antigens with distinct antigenicity to avoid host immune destruction. This permits repeated “waves” of parasitemia or bacteremia. Hitherto, this phenomenon has not been reported in Lyme disease. Although the genetic mechanism of variation is similar, by gene conversion, in *T. brucei* (9, 10, 35), *B. hermsii* (25, 33), *A. marginale* (28), and *B. burgdorferi* (36–38), the effect of antigenic variation may be different. VSG, Vmp, and MSP-2 (major surface protein 2 of *A. marginale*) may use their immunodominant variable domains to divert the antibody response from their conserved portions (9, 10, 28, 33, 35), while VlsE may employ its immunodominant conserved portions to suppress the immune response to other membrane targets. The C-terminal domain of VlsE, like IR₂ and IR₆, in view of its immunodominance, may provide such a decoy effect. Perhaps, as a consequence, it must remain unavailable to antibody during infection. Thus, the C-terminal domain of VlsE may not serve as target for a protective immune response against *B. burgdorferi*.

ACKNOWLEDGMENTS

This work was supported in part by grant RR00164 from the NCI, National Institutes of Health.

The technical skills of Charles Garret are gratefully acknowledged.

REFERENCES

- Akin, E., G. L. McHugh, R. A. Flavell, E. Fikrig, and A. C. Steere. 1999. The immunoglobulin (IgG) antibody response to OspA and OspB correlates with severe and prolonged Lyme arthritis and the IgG response to P35 correlates with mild and brief arthritis. *Infect. Immun.* **67**:173–181.
- Akins, D. R., K. W. Bourell, M. J. Caimano, M. V. Norgard, and J. D. Radolf. 1998. A new animal model for studying Lyme disease spirochetes in a mammalian host-adapted state. *J. Clin. Invest.* **101**:2240–2250.
- Barbet, A. F., and T. C. McGuire. 1978. Crossreacting determinants in variant-specific surface antigens of African trypanosomes. *Proc. Natl. Acad. Sci. USA* **75**:1989–1993.
- Barony, G., and R. B. Merrifield. 1980. The peptides: analysis, synthesis, & biology, p. 3–285. Academic Press, New York, N.Y.
- Barstad, P. A., J. E. Coligan, M. G. Raum, and A. G. Barbour. 1985. Variable major proteins of *Borrelia hermsii*, epitope mapping and partial sequence analysis of CNBr peptides. *J. Exp. Med.* **161**:1302–1314.
- Barthold, S. W., and L. K. Bockenstedt. 1993. Passive immunizing activity of sera from mice infected with *Borrelia burgdorferi*. *Infect. Immun.* **61**:4696–4702.
- Benjamin, D. C. 1995. B-cell epitopes: fact and fiction, p. 95–108. In L. M. Aledort et al. (ed.), *Inhibitors to coagulation factors*. Plenum Press, New York, N.Y.
- Berzofsky, J. A., and I. J. Berkower. 1993. Immunogenicity and antigen structure, p. 235–282. In W. E. Paul (ed.), *Fundamental immunology*. Raven Press, Ltd., New York.
- Borst, P. 1991. Molecular genetics of antigenic variation. *Immunol. Today* **12**:A29–A33.
- Borst, P., and G. A. M. Cross. 1982. Molecular basis for trypanosome antigenic variation. *Cell* **29**:291–303.
- Cross, G. A. M. 1979. Crossreacting determinants in the C-terminal region of trypanosome variant surface antigens. *Nature* **277**:310–312.
- Escudero, R., M. L. Halluska, P. B. Backenson, J. L. Coleman, and J. L. Benach. 1997. Characterization of the physiological requirements for the bactericidal effects of a monoclonal antibody to OspB of *Borrelia burgdorferi* by confocal microscopy. *Infect. Immun.* **65**:1908–1915.
- Feldman, D. E., and J. Frydman. 2000. Protein folding in vivo: the importance of molecular chaperones. *Curr. Opin. Struct. Biol.* **10**:26–33.
- Fikrig, E., S. W. Barthold, F. S. Kantor, and R. A. Flavell. 1990. Protection of mice against the Lyme disease agent by immunizing with recombinant OspA. *Science* **250**:553–556.
- Gilmore, R. D., Jr., K. J. Kappel, M. C. Dolan, T. R. Burkot, and B. J. Johnson. 1996. Outer surface protein C (OspC), but not P39, is a protective immunogen against a tick-transmitted *Borrelia burgdorferi* challenge: evidence for a conformational protective epitope in OspC. *Infect. Immun.* **64**:2234–2239.
- Kochi, S. K., and R. C. Johnson. 1988. Role of immunoglobulin G in killing of *Borrelia burgdorferi* by the classical complement pathway. *Infect. Immun.* **56**:314–321.
- Kochi, S. K., R. C. Johnson, and A. P. Dalmasso. 1991. Complement-mediated killing of the Lyme disease spirochete *Borrelia burgdorferi*: role of antibody in formation of an effective membrane attack complex. *J. Immunol.* **146**:3964–3970.
- Kochi, S. K., R. C. Johnson, and A. P. Dalmasso. 1993. Facilitation of complement-dependent killing of the Lyme disease spirochete, *Borrelia burgdorferi*, by specific immunoglobulin G Fab antibody fragments. *Infect. Immun.* **61**:2532–2536.
- Liang, F. T., A. L. Alvarez, Y. Gu, J. M. Nowling, R. Ramamoorthy, and M. T. Philipp. 1999. An immunodominant conserved region within the variable domain of VlsE, the variable surface antigen of *Borrelia burgdorferi*. *J. Immunol.* **163**:5566–5573.
- Liang, F. T., and M. T. Philipp. 1999. Analysis of antibody response to invariable regions of VlsE, the variable surface antigen of *Borrelia burgdorferi*. *Infect. Immun.* **67**:6702–6706.
- Liang, F. T., A. C. Steere, A. R. Marques, B. J. B. Johnson, J. N. Miller, and M. T. Philipp. 1999. Sensitive and specific serodiagnosis of Lyme disease by ELISA with a peptide based on an immunodominant conserved region of *Borrelia burgdorferi* VlsE. *J. Clin. Microbiol.* **37**:3990–3996.
- Liang, F. T., J. M. Nowling, and M. T. Philipp. 2000. Cryptic and exposed invariable regions of VlsE, the variable surface antigen of *Borrelia burgdorferi* sl. *J. Bacteriol.* **182**:3597–3601.
- Liang, F. T., R. H. Jacobson, R. K. Straubinger, A. Grooters, and M. T. Philipp. 2000. Characterization of a *Borrelia burgdorferi* VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* **38**:4160–4166.
- Ma, J., C. Gingrich-Baker, P. M. Franchi, P. Bulger, and R. T. Coughlin. 1995. Molecular analysis of neutralizing epitopes on outer surface proteins A and B of *Borrelia burgdorferi*. *Infect. Immun.* **63**:2221–2227.
- Meier, J. T., M. I. Simon, and A. G. Barbour. 1985. Antigenic variation is associated with DNA rearrangements in a relapsing fever *Borrelia*. *Cell* **41**:403–409.
- Montgomery, R. R., S. E. Malawista, K. J. M. Feen, and L. K. Bockenstedt. 1996. Direct demonstration of antigenic substitution of *Borrelia burgdorferi* ex vivo: exploration of the paradox of the early immune response to outer surface proteins A and C in Lyme disease. *J. Exp. Med.* **183**:261–269.
- Nowling, J. M., and M. T. Philipp. 1999. Killing of *Borrelia burgdorferi* by antibody elicited by OspA vaccine is inefficient in the absence of complement. *Infect. Immun.* **67**:443–445.
- Palmer, G. H., W. C. Brown, and F. R. Rurangirwa. 2000. Antigenic variation in the persistence and transmission of the ehrlichia *Anaplasma marginale*. *Microbes Infect.* **2**:167–176.
- Philipp, M. T., M. K. Aydin, R. P. Bohm, Jr., F. B. Cogswell, V. A. Dennis, H. N. Lanners, R. C. Lowrie, Jr., E. D. Roberts, M. D. Conway, M. Karaçorlu, G. A. Peyman, D. J. Gubler, B. J. B. Johnson, J. Piesman, and Y. Gu. 1993. Early and early disseminated phases of Lyme disease in the rhesus monkey: a model for infection in humans. *Infect. Immun.* **61**:3047–3059.
- Schwan, T. G., J. Piesman, W. T. Golde, M. C. Dolan, and P. A. Rosa. 1995. Induction of an outer surface protein on *Borrelia burgdorferi* during tick feeding. *Proc. Natl. Acad. Sci. USA* **92**:2909–2913.
- Schwan, T. G., and J. Piesman. 2000. Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, *Borrelia burgdorferi*, during the chain of infection in ticks and mice. *J. Clin. Microbiol.* **38**:382–388.
- Scriba, M., J. S. Ebrahim, T. Schlott, and H. Eifert. 1993. The 39-kilodalton protein of *Borrelia burgdorferi*: a target for bactericidal human monoclonal antibodies. *Infect. Immun.* **61**:4523–4526.
- Stoenner, H. G., T. Dodd, and C. Larsen. 1982. Antigenic variation of *Borrelia hermsii*. *J. Exp. Med.* **156**:1297–1311.
- Towbin, H., T. Staechelin, and J. Gordon. 1979. Electrophoretic transfer of

- proteins from polyacrylamide gels to introcellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350–4354.
35. **Van der Ploeg, L. H. T., K. Gottesdiener, and M. G. S. Lee.** 1992. Antigenic variation in African trypanosomes. *Trends Genet.* **8**:452–457.
36. **Zhang, J. R., J. M. Hardham, A. G. Barbour, and S. J. Norris.** 1997. Antigenic variation in Lyme disease borreliae by promiscuous recombination of VMP-like sequence cassettes. *Cell* **89**:275–285.
37. **Zhang, J. R., and S. J. Norris.** 1998. Genetic variation of the *Borrelia burgdorferi* gene *vlsE* involves cassette-specific, segmental gene conversion. *Infect. Immun.* **66**:3698–3704.
38. **Zhang, J. R., and S. J. Norris.** 1998. Kinetics and in vivo induction of genetic variation of *vlsE* in *Borrelia burgdorferi*. *Infect. Immun.* **66**:3689–3697.

Editor: V. J. DiRita