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Intragenic Recombination and a Chimeric Outer Membrane Protein in the Relapsing Fever Agent *Borrelia hermsii*

TODD KITTEN, ADRIAN V. BARRERA, AND ALAN G. BARBOUR*

Departments of Microbiology and Medicine, University of Texas Health Science Center, San Antonio, Texas 78284-7758

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The spirochete *Borrelia hermsii*, a relapsing fever agent, evades the host's immune response through multiphasic antigenic variation. Antigen switching results from sequential expression of genes for serotype-specific outer membrane proteins known as variable major proteins (Vmp's); of the 25 serotypes that have been identified for the HS1 strain, serotypes 7 and 21 have been studied in greatest detail. In the present study, an atypical variant was predominant in the relapse from a serotype 21 infection in mice; relapse cells were bound by monoclonal antibodies specific for Vmp21 as well as antibodies specific for Vmp7. In Western blots (immunoblots), the variant had a single Vmp that was reactive with monoclonal antibodies representing both serotypes. The gene encoding this Vmp, *vmp7/21*, was cloned and characterized by restriction mapping and sequence analysis to determine the likely recombination event. Whereas the 5′ end of *vmp7/21* was identical to that of *vmp21*, its 3′ end and flanking sequences were identical to the 3′ end of *vmp7*. Unlike other *vmp* genes examined thus far, the *vmp7/21* gene existed only in an expressed form; a silent, storage form of the gene was not detected. We conclude that the *vmp7/21* gene was created by an intragenic recombination between the formerly expressed *vmp21* gene and a silent *vmp7* gene. This finding suggests that the lack of cross-reactivity between variants, which is usually observed, results from immunoselection against variants possessing chimeric Vmp's rather than from a switching mechanism that excludes partial gene replacements.

The genus *Borrelia* is in the eubacterial phylum of spirochetes and includes the etiologic agents of relapsing fever, avian spirochetosis, and Lyme disease (10, 29). The course of relapsing fever is characterized by episodes of fever and bacteremia separated by periods of well-being. Relapsing fever-causing borrelias evade the mammalian host's immune response through multiphasic antigenic variation (5, 35). Antigen switching is the consequence of sequential expression of genes for serotype-specific outer membrane proteins known as variable major proteins (Vmp's) (6, 13, 27, 31). Twenty-five different variants or serotypes have been identified among the progeny of a single cell of the HS1 strain of *Borrelia hermsii* (35).

Serotypes 7 and 21 have been studied in greatest detail. The Vmp's expressed by these cells, Vmp7 and Vmp21, have distinctive peptide maps and monoclonal and polyclonal antibody reactivities (6, 13, 15) despite 77% nucleotide sequence identity of their genes (17). Western blot (immunoblot) analysis of peptides generated by cyanogen bromide cleavage and secondary structure predictions by algorithm indicate that serotype-specific epitopes are distributed throughout the proteins (15, 17).

Whereas cells of both serotypes contain Vmp7 and Vmp21 genes (*vmp7* and *vmp21*) that are not transcribed, serotype 7 cells contain a unique expressed version of *vmp7* and serotype 21 cells, likewise, contain a unique expressed *vmp21* (27). Silent and expressed *vmp* genes are located on separate linear plasmids (31). The 3′ ends of expressed *vmp7* and *vmp21* genes are usually within 1 to 1.5 kb from the telomeres of 28-kb plasmids (22). These expression plasmids, designated bp7E and bp21E, respectively, are identical in sequence 5′ to the *vmp* genes, and both contain a consensus σ^{70}-type promoter. Transcription from *vmp7* has been mapped to the consensus promoter (7). Downstream from the *vmp* genes, the terminal 300-bp sequences of these expression plasmids are also virtually identical. The silent *vmp* genes are identical to their expressed counterparts but lack their consensus promoter; their 3′ ends are located 4 to 6 kb from silent plasmid telomeres (7, 17, 22). A 200-bp block of sequence identity, known as the downstream homology sequence (DHS), is located about 1 kb downstream from the expressed and silent *vmp7* and *vmp21* genes (22). A similar arrangement has been found for four other expressed *vmp* genes that have been examined (32).

These findings suggested that a switch from expression of one Vmp to another is the result of a nonreciprocal recombination between linear plasmids bearing the expressed *vmp* and silent *vmp* genes (5, 22, 31). According to this model, a complete copy of a silent *vmp* and a certain length of 3′-flanking DNA displace the expressed *vmp* gene. The upstream recombination site is located within a 50-bp region of homology containing the beginning of the *vmp*, while the 3′ recombination occurs within the DHS. As an exception to this mechanism, we report here an incomplete switch resulting in a chimeric Vmp protein. Progeny of this switch produced a Vmp containing epitopes found in both Vmp7 and Vmp21. This novel Vmp was created by partial replacement of the expressed *vmp21* gene with a silent *vmp7*. The recombination sites for this switch were identified and compared with sites used in a complete switch between serotypes 7 and 21.

MATERIALS AND METHODS

Bacterial strains and plasmids. Origins of *B. hermsii* HS1 (ATCC 35209) and 25 of its isogenic serotypes are specified in references 13 and 35; the serotype 21 cells used in this study were derived from serotype 7. Borrelias were propa-

* Corresponding author. Electronic mail address: alan@taliesin.uthscsa.edu.

TABLE 1. Reactivities of monoclonal antibodies with serotype 7/21 cells by indirect immunofluorescence

Antibody(ies)	Specificity	% Thick cells with blebs	% Thin cells without blebs
H10022	Vmp21	100	0
H4116	Vmp21	100	0
H10211	Vmp21	0	0
H12135	Vmp21, Vmp7	100	0
H12936	Vmp7	100	0
H9724	Flagellin	0	100
H10022 + H9724	Vmp21, flagellin	90	10
H12135 + H9724	Vmp7, Vmp21, flagellin	90	10
H12936 + H9724	Vmp7, flagellin	90	10

gated in mice irradiated by using a Cs^{137} source (35) or in BSK II medium and frozen at −80°C until use (2). Cloning of cells by limiting dilution in mice was performed as described previously (35). Recombinant plasmids containing expressed *vmp21* (p21.4), silent *vmp21* (p7.31), and silent *vmp7* (p7.12) of *B. hermsii* HS1 were described previously (31).

Immunofluorescence and Western blot analysis. Borrelias were examined by indirect immunofluorescence; staining reactions were categorized as negative, positive with thick cells and blebs, or positive with thin cells and no blebs. The last reaction is characteristic of antibodies recognizing internal antigens, such as the periplasmic flagella (11, 35). Antibodies producing stained borrelias that are thick and have membrane blebs have specificities for outer membrane antigens, such as the Vmp proteins (3, 25). Whole-cell lysates were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and examined by Western blot as described previously (12, 13). Anti-Vmp monoclonal antibodies H10022, H4116, H10211, H12135, H12915, and H12936 have been described previously (3, 4, 15); monoclonal antibody H9724 is directed against a flagellar epitope common to *Borrelia* spp. (11). Specificities of antibodies used in this study are given in Table 1; antibody H12915 is specific for serotype 7 (15).

DNA procedures. Plasmid-enriched DNA was isolated from *B. hermsii* by the method of Barbour and Garon (8). Plasmid DNA from *Escherichia coli* was extracted by the alkaline lysis method (26) and purified by CsCl density centrifugation. Restriction sites were located by standard techniques, including partial digestion of end-labeled DNA (34). Total borrelial DNA, which had been partially digested with *Sau*3AI, was cloned into the bacteriophage vector λEMBL3 as described previously (31). For Southern blot analysis, restriction fragments were electrophoretically separated on agarose gels in 90 mM Tris-90 mM borate-2 mM EDTA, pH 8.0, containing 0.2 μg of ethidium bromide per ml, partially acid depurinated, denatured under alkaline conditions, and transferred to 0.45-μm-pore-size nylon membranes (Nytran; Schleicher and Schuell, Manchester, N.H.). Oligonucleotide probes were radiolabeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (26) and purified by passage through Nensorb 20 cartridges (DuPont, Boston, Mass.). Hybridization of radiolabeled probes with the membranes was in 0.1% SDS–10× Denhardt's solution–4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1 mg of denatured salmon sperm DNA per ml (26). The membranes were washed at 37°C in 6× SSC–0.1% SDS.

DNA sequence analysis. The inserts of recombinant plasmids were sequenced by double-stranded dideoxy sequencing (18) with Sequenase (U.S. Biochemical Corp., Cleveland, Ohio). The polymerase chain reaction (PCR) was used to amplify genomic DNA from borrelias for sequencing (21). Initial amplifications were performed in 100-μl reaction mixtures containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$–0.001% gelatin, 0.2 mM deoxynucleoside triphosphates, 2.5 U of *Thermus aquaticus* DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.), 25 ng of serotype 7/21 DNA, and 50 pmol each of the two oligonucleotide primers. Samples were overlaid with 50 μl of mineral oil and heated in a PTC-100 thermal cycler (MJ Research, Cambridge, Mass.) to 94°C for 1.5 min for initial denaturation, and then they were cycled 30 times at 94°C for 1 min, 55°C for 1 min, and 72°C for 3 min. The last cycle was followed by an additional 7 min at 72°C. PCR products were recovered by electrophoresis and electroelution. Single-stranded DNA was generated from these products by the same procedure, except that only one amplification primer was used. The recovered single-stranded DNA was sequenced with Sequenase and specific oligonucleotide primers. Custom oligonucleotide primers for sequencing and PCR were synthesized on an Applied Biosystems synthesizer on the basis of sequence information reported here and by Burman et al. (17). The primers used for amplification were 5′-CCCTAAA GATGAAACTATAGCAAG-3′ (primer 1) and 5′-CCCCT TGCCGCTTCCCCAGT-3′ (primer 2). Primer 1 binds to the upstream expression region, at positions −125 to −148 relative to the protein start site of *vmp7* and *vmp21* (7, 17). Primer 2 binds in the downstream conserved telomere, 2 to 2.5 kb from the start site of *vmp7* and *vmp21*.

RESULTS

A single Vmp contains epitopes of both Vmp7 and Vmp21. Approximately 10^3 serotype 21 cells were injected intraperitoneally into adult BALB/c mice that had inadvertently received a whole-body dose of γ-irradiation estimated to be 300 to 350 rads instead of the usual 650 rads (15, 35). During the first relapse of spirochetemias in the mice, blood was drawn and used to infect a second group of mice that had been fully irradiated with 650 rads. During the early spirochetemia of the second group of mice, blood was drawn and inoculated into broth medium.

A culture from one mouse was subsequently examined by an indirect immunofluorescence assay with monoclonal antibodies directed against different serotype-specific and genus-specific epitopes. The results of this analysis are summarized in Table 1. Both of the monoclonal antibodies that are specific for Vmp7 epitopes and two of three antibodies that recognize Vmp21 epitopes bound to cells of this isolate and gave an immunofluorescence reaction typical of antibodies binding to outer membrane-associated antigens. Antibody H12135, which binds to a shared epitope on Vmp7 and 21 (3), also bound to cells in this way.

Because it was possible that this relapse population was a mixture of two or more different serotypes, some smears were reacted with a Vmp-specific antibody and an antiflagellin antibody simultaneously. This experiment showed that each of the Vmp-specific monoclonal antibodies accounted for at least 90% of the cells that were present. Only 10% or fewer of the borrelias in the double-staining study gave a reaction typical of the antiflagellin and genus-specific antibody H9724. When H9724 was used alone, 100% of the fluorescent cells had this reaction; the outer membrane-type reaction dominated the less prominent antiflagellin reaction when both types of antibody were present.

We concluded that at least 90% of the cells in the popu-

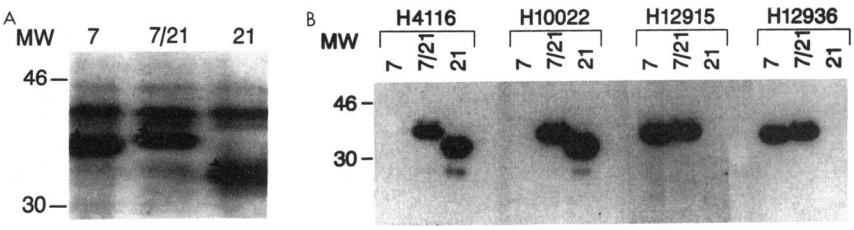

FIG. 1. Comparison of Vmp's from serotypes 7, 7/21, and 21 of *B. hermsii* HS1. (A) Coomassie blue-stained proteins in whole lysates subjected to SDS-PAGE. The relative migration of selected molecular weight (MW) standards (whose MWs in thousands are given on the left) is shown. Vmp proteins are indicated (arrowheads). (B) Western blot analysis with Vmp21- and Vmp7-specific monoclonal antibodies H4116, H10022, H12915, and H12936.

lation were recognized by monoclonal antibodies specific for either Vmp7 or Vmp21 and that this majority population represented a single cell type or serotype. To obtain a purer population of the chimeric borrelia, the population of spirochetes was cloned by limiting dilution in mice (35). When smears of blood from mice inoculated with the highest dilution that was still infectious were examined, all cells present gave the outer membrane-type reaction when examined with the same monoclonal antibodies (not shown).

This cloned population was cultured for approximately 12 generations in broth medium, and the harvest was examined by SDS-PAGE and Western blot analysis. Fig. 1A shows that there is only one Vmp protein in the chimeric variant. This Vmp had an apparent size of 38.5 kDa, compared with apparent sizes of 38.0 kDa for Vmp7 and 36.0 kDa for Vmp21. The major protein of 41 kDa in Fig. 1A is flagellin (11). Figure 1B shows the reactions of representative monoclonal antibodies against these Vmps. This experiment demonstrated that the chimeric borrelia had a single Vmp protein that appeared to account for the binding of the serotype 7- and 21-specific monoclonal antibodies. The reactivities of the antibodies listed in Table 1 and shown in Fig. 1B have been determined against cyanogen bromide fragments of Vmp7 and Vmp21 (4, 15). Antibody H10211 recognizes the CB5 carboxy-terminal CNBr fragment of Vmp21; the epitope for this antibody is not in Vmp7/21. Chimera-reactive antibodies H4116 and H10022 bind to epitopes assigned to the first (CB3) and second (CB4) CNBr fragments of Vmp21, respectively (4, 15). Antibodies H12936 and H12915, which also bind to Vmp7/21, bind to one or the other of the two CNBr fragments (CB1 and CB2) of Vmp7 (4, 15; also, unpublished observations). In summary, the chimeric protein contained serotype-specific epitopes from both CNBr fragments of Vmp7 and from two of three CNBr fragments of Vmp21.

The *vmp7/21* gene occupies the expression locus in chimeric cells. The active *vmp* gene in the chimeric cells was isolated and compared with the expressed *vmp7* and *vmp21* (17, 31). Most copies of expressed *vmp* genes occupy a conserved expression locus, bounded upstream by a homology block and promoter and downstream by a different homology block and a telomere (7, 22, 32). We assumed that the putative *vmp7/21* gene occupied this site also, and hence, the expression locus from serotype 7/21 cells was cloned. A restriction fragment specific for the region upstream of active *vmp* genes was used to probe a λEMBL3 bank of total DNA extracted from serotype 7/21 cells (31). Previous studies had shown that Vmp7 and Vmp21 are expressed in *E. coli* from recombinant phage and plasmids if the cloned genes are taken from the expression locus (7, 31). Therefore, hybridizing recombinant phages were examined by plaque immunoblot for reactivities with the serotype 7- and serotype 21-specific antibodies. All hybridizing plaques, like the variant borrelias themselves, were bound by both serotype 7-specific antibodies and by two of three serotype 21-specific antibodies. An 8.5-kb *Bam*HI fragment from the recombinant phage was subcloned into vector pBR322. This recombinant plasmid was designated pBOR5. Figure 2 shows the reactivities of three monoclonal antibodies with lysates of serotype 7/21 organisms and lysates of *E. coli* containing pBOR5. The Western blot indicates that pBOR5 produces a protein in *E. coli* that is identical in antibody reactivity and electrophoretic mobility to Vmp7/21. Lysates from *E. coli* containing pBR322 showed no reactivity to any of the three monoclonal antibodies (not shown).

***vmp7/21* is a hybrid of *vmp21* and *vmp7*.** A restriction map of the recombinant plasmid was created. *B. hermsii* DNA downstream from that which had been cloned was examined by Southern blot analysis of linear plasmid DNA. The location of the downstream telomere was determined by Southern blot analysis of *Bal* 31-digested DNA (9, 20, 22). Figure 3 shows the restriction map of the expression locus in serotype 7/21 compared with the expression loci in serotypes 7 and 21, which had been mapped previously (7, 22, 31). The plasmid bearing the expression locus from serotype 7/21 was designated bp7/21E, because it resembles expression plasmids bp7E and bp21E in having a telomeric *vmp* gene. The restriction maps of the right ends of bp7/21E and bp7E were identical with the exception of a *Rsa*I cleavage site near the beginning of what would be the expressed *vmp* in bp7/21E. This *Rsa*I site was in the same location as the first *Rsa*I site of *vmp21*. It thus appeared that the expression locus in serotype 7/21 contained a short region of *vmp21*-homologous sequence followed by a longer region homologous to *vmp7* and its 3'-flanking sequence.

To confirm this assessment and to determine whether the

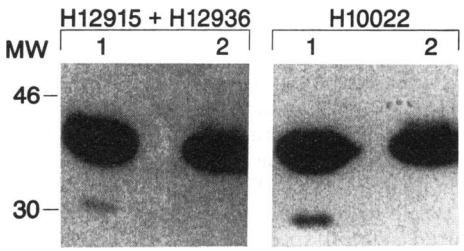

FIG. 2. Western blot analysis of lysates of *B. hermsii* serotype 7/21 (lane 1) and *E. coli* containing pBOR5 (lane 2). Monoclonal antibodies H12915 and H12936 are specific for Vmp7; H10022 is specific for Vmp21.

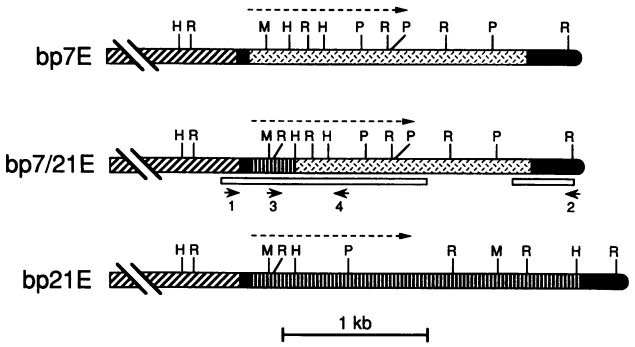

FIG. 3. Restriction maps of expression loci of three serotypes of *B. hermsii*. The right ends of the predominant expression plasmids from serotype 7 (bp7E), serotype 7/21 (bp7/21E), and serotype 21 (bp21E) are shown. Dashed arrows indicate the locations and orientations of expressed *vmp* genes. The black boxes are the homology block around the 5′ ends of the *vmp* genes; the gray boxes are the DHS and the telomere (22). The hatched area is the upstream region common to all expression plasmids. Boxes with vertical lines indicate the *vmp21* sequence, and boxes with partial crosshatched lines indicate the *vmp7* sequence. Open boxes underneath bp7/21E indicate those regions that were sequenced for this study. Numbered arrows depict binding sites for oligonucleotides used as probes for Southern blots or as PCR primers. Restriction enzymes: H, *Hin*dIII; M, *Msp*I; P, *Pst*I; R, *Rsa*I.

expression site in 7/21 cells encoded Vmp7/21, DNA of pBOR5 was sequenced; the region sequenced is indicated by the leftmost open bar under bp7/21E in Fig. 3. The sequence of 200 bp upstream of the *vmp* gene was determined and found to be identical to the sequence immediately upstream of *vmp7* and *vmp21* (7). This sequence includes the homopolymeric stretch of 16 T's, the consensus σ^{70} promoter elements, the CAT mRNA start site, and the probable ribosome binding site (7, 17). An open reading frame, the first 300 bp of which were identical to *vmp21*, followed (17). The sequence then became identical to *vmp7* (17) and remained so for at least 50 bp past the end of the open reading frame. Figure 4 shows aligned partial nucleotide sequences of *vmp7*, *vmp21*, and *vmp7/21*. The juncture between the *vmp21*- and *vmp7*-like regions in *vmp7/21* is underlined.

The open reading frame of bp7/21E would encode a protein 250 Da larger than Vmp7; this was consistent with the results shown in Fig. 1. On the basis of the antibody

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7     TTAGTGTCAGATGTGTTGGGATTTACTGCAAAATCAGATACAACTAAGCAAGAAGTAGGA 258
7/21  TTAGTTTCAGATACATTAGGCTTTACTGCTAAATCAACTACAAAGAAAGAGGATGTAGGG 267
21    TTAGTTTCAGATACATTAGGCTTTACTGCTAAATCAACTACAAAGAAAGAGGATGTAGGG 267

7     GGTTATTTTAACAGCCTAGGTGCGAAGCTTGGAGAGGCGTCAAATGACTTGGAACAAGTA 318
7/21  GGTTATTTTAACAGCCTGGGTGGTAAGCTTGGAGAGGCGTCAAATGACTTGGAACAAGTA 327
21    GGTTATTTTAACAGCCTGGGTGGTAAGCTTGGAGAAGCATCAAATGAGTTAGAACAAGTA 327

7     GCAGTAAAAGCAGAAACAGGTGTTGATAAAAGCGATTCATCAAAAAATCCAATTAGAGAA 378
7/21  GCAGTAAAAGCAGAAACAGGTGTTGATAAAAGCGATTCATCAAAAAATCCAATTAGAGAA 387
21    GCAAAAAATTCAGAAGCAGGCATTGAAAAAAATGACGCATCAAAAAATCCAATTAGAAGT 387
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FIG. 4. Partial nucleotide sequences of *vmp7* (7), *vmp7/21* (7/21), and *vmp21* (21). *vmp7* and *vmp21* sequences are from reference 17. Numbers indicate distances in base pairs from the start of translation from reference 7. Vertical bars indicate sequence identity. The region where *vmp7/21* switches from identity with *vmp21* to identity with *vmp7* is underlined.

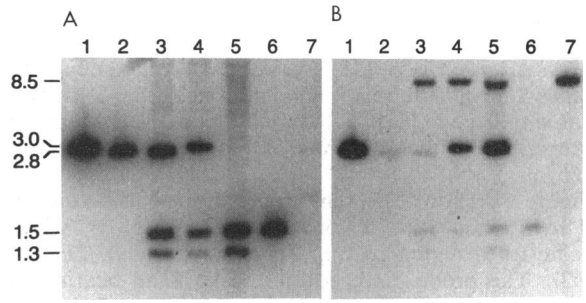

FIG. 5. Southern blot analysis of *B. hermsii* DNA with probes specific for *vmp21* and *vmp7*. *Pst*I-digested DNA samples are shown as follows: lane 1, pBOR5; lane 2, p21.4 (31), containing expressed *vmp21*; lane 3, serotype 21 borrelias; lane 4, serotype 7/21 borrelias; lane 5, serotype 7 borrelias; lane 6, p7.31 (31), containing silent *vmp21*; lane 7, p7.12 (31), containing silent *vmp7*. The molecular sizes (in kilobases) of the hybridizing *Pst*I fragments are shown on the left. (A) Blot hybridized with oligonucleotide 3, which is specific for *vmp21*; (B) same blot stripped of the first probe and hybridized with oligonucleotide 4, which is specific for *vmp7*.

reactivities of the phage clone and pBOR5 and the location, size, and sequence of the open reading frame, we concluded that we had identified the expression site for *vmp7/21*, the position of which is indicated in Fig. 3.

We next examined DNA further downstream from *vmp7/21* to confirm whether it was identical with DNA distal to *vmp7*. We amplified 7/21 DNA by the PCR and sequenced the products (21). Primers 1 and 2 (Fig. 3) were synthesized to bind to the conserved upstream expression site and the conserved downstream telomere, respectively (5, 22), and in a preliminary study they amplified the *vmp* genes from bp7E and bp21E. When these primers were used to amplify the expression site of bp7/21E, a product of the expected size was obtained. Approximately 400 bp of this product was sequenced from this telomeric region of bp7/21E (Fig. 3). The sequence was identical to that of bp7E (22, 32). To exclude the possibility that we had amplified and sequenced contaminating bp7E DNA rather than bp7/21E DNA, we also sequenced the expected *vmp21-vmp7* juncture region of the amplified product. Approximately 400 bp of sequence was obtained by using an oligonucleotide primer that binds in the *vmp7*-like region of *vmp7/21*. The sequence spanned the entire region shown in Fig. 4 and was identical to the *vmp7/21* sequence obtained from the recombinant plasmids. These findings indicated that we had amplified and sequenced serotype 7/21 DNA and confirmed the identity of sequences 3′ to the *vmp* gene in bp7/21E and bp7E.

***vmp7/21* was created by intragenic recombination at the expression site.** Two possible explanations for our findings were apparent: the *vmp21* gene resident in the expression site was either partially replaced by a silent *vmp7* gene or completely replaced by a silent *vmp7/21* gene. To decide between these models, evidence for a silent *vmp7/21* gene was sought. Figure 5A shows a Southern blot of *Pst*I-digested DNA from serotype 21, serotype 7/21, and serotype 7 organisms probed with an oligonucleotide representing a region common to both *vmp21* and *vmp7/21* (oligonucleotide 3 in Fig. 3); the blot also contains DNA from recombinant plasmids containing expressed *vmp7/21*, expressed *vmp21*, silent *vmp21*, and silent *vmp7*. The probe hybridized to *Pst*I fragments of four sizes. (i) A 3.0-kb fragment is present in lanes 1 and 4; this fragment contains the 5′ end of the

expressed *vmp7/21* gene. (ii) A 2.8-kb fragment is present in lanes 2 and 3; this fragment contains the 5′ end of the expressed *vmp21* gene (31). (iii) A 1.5-kb fragment is present in lanes 3 through 6; this fragment contains the 5′ end of the silent *vmp21* gene (31). (iv) An unidentified 1.3-kb fragment is present in genomic DNAs from all three serotypes (lanes 3 to 5).

Figure 5B shows the same blot stripped of bound probe and hybridized with oligonucleotide 4 (Fig. 3), which is specific for a region of *vmp7* common to *vmp7/21* but not *vmp21*. The probe binds to the 8.5-kb band bearing silent *vmp7* present in all three serotypes (lanes 3 to 5) and in the recombinant plasmid containing silent *vmp7* (lane 7). The 3.0-kb fragment containing expressed *vmp7/21* (lanes 1 and 4) or *vmp7* (lane 5) also hybridizes with probe 4. If a silent *vmp7/21* gene exists, it should reside on a *Pst*I fragment that hybridizes to both probes used. No such fragment was evident. Although we cannot exclude the possibility of a silent *vmp7/21* gene, residing uniquely in serotype 7/21 cells on a 3.0-kb fragment that comigrated with the fragment containing expressed *vmp7/21*, this seems unlikely. Scanning densitometry confirmed that the 3.0-kb band in lane 4 has about the same intensity relative to other bands in the lane as do the 3.0- and 2.8-kb bands in serotypes 7 and 21 (data not shown), a finding that would not be expected if the 3.0-kb band in serotype 7/21 contained both silent and expressed *vmp7/21* genes.

DISCUSSION

We have analyzed a chimeric Vmp using monoclonal antibodies and characterized its gene by molecular cloning, restriction site analysis, and DNA sequencing. Antibody reactivities indicated that Vmp7/21 contained epitopes found on both CNBr fragments of Vmp7 (CB1 and CB2) and on CNBr fragments CB3 and CB4 but not CB5 of Vmp21 (4, 15). The sequence of *vmp7/21* indicates that its protein product contains at least parts of CB1, CB2, and CB3 and, thus, is in agreement with the antibody results. However, CB4 could not be encoded by the *vmp7/21* gene; it is located in the middle of Vmp21, past the juncture point in the chimeric protein. This discrepancy is accountable by a mistaken assignment of antibody H10022 reactivity to CB4 (15). Sequence analysis of *vmp7* and *vmp21* indicates that CNBr cleavage of the protein products should produce fragments of 2.4 kDa upstream from CB1 in Vmp7 and of 2.6 kDa upstream from CB3 in Vmp21 (17). These fragments were not detected in previous studies, probably because the first residue of each is blocked by an attached lipid (17). It is likely that H10022 reacts to an epitope on the 2.6-kDa peptide fragment shared by Vmp21 and Vmp7/21 rather than the 5.6-kDa CB4 found only in Vmp21.

When Stoenner and colleagues isolated and characterized 25 serotypes of *B. hermsii*, the only evidence of a chimeric Vmp was an occasional relapse isolate that reacted with polyclonal antiserum to serotype 16 as well as to serotype 7 (12, 35). None of the hundreds of relapse isolates that were typed with a battery of serotype-specific antisera resembled the 7/21 cells studied here. However, the 7/21 hybrid described here probably would not have been discovered by using the isolation protocol of Stoenner et al. (35). New serotypes were isolated by infecting an immunocompetent mouse with a clonal population of borrelias, allowing for clearance of the infecting serotype present in the first spirochetemia, and then collecting organisms from the second or relapse spirochetemia. Circulating antibodies to the infecting serotype, serotype 21 in the present case, likely prevented partial antigenic switches. Once obtained, the relapse populations were maintained in mice that were immunosuppressed with cyclophosphamide or 650 to 900 rads of gamma irradiation (15, 35).

The mice from which 7/21 was isolated had received 300 to 350 rads, a dose of gamma radiation that has been shown to partially prevent the primary antibody response to an antigenic challenge in mice (1). The neutralizing antibody response directed against *B. hermsii* and another relapsing fever species, *Borrelia turicatae*, appears to be T-cell independent (28, 35). A study by Lee and Woodland (25) would suggest that a dose of 300 to 350 rads of whole-body irradiation given to a BALB/c mouse would eliminate only about half of the B cells responding to thymus-independent antigens. Thus, these borrelia-infected mice likely responded with an incomplete antibody repertoire. The antibodies produced may have been sufficient to eliminate the infecting serotype 21 organisms but not the variant displaying the hybrid Vmp.

Past studies have shown that the host antibody response eliminates those organisms that do not switch but is not required for the switch phenomenon, which can occur in vitro in the absence of specific antibody (35). This study suggests that the antibody response may also eliminate organisms that undergo incomplete conversions of the expressed *vmp* gene. In an attempt to determine the frequency with which hybrid genes are formed, PCR was used to amplify additional *vmp7/21* genes from populations of serotype 21 cells. Although genuine hybrids existing in the population of borrelias could not be detected above the background of artifactual hybrids created during amplification, this in vitro finding indicated that the substrates were sufficient for intragenic recombinations (unpublished findings).

A relapse population expressing a hybrid Vmp has implications for models of *vmp* gene switching. In a switch from Vmp7 to Vmp21 during an infection in mice, two recombination sites were involved: the 5′ site in a 50-bp region of homology encompassing the beginning of *vmp* genes and the 3′ site within the DHS (22). In the switch examined here, the 3′ recombination occurred within the DHS as expected, but the upstream recombination site was 300 bp distal to the 5′ ends of the genes. This new upstream recombination site was not similar to recombination sites identified previously (22, 32). These findings suggest at least two recombination models, each of which could operate by gene conversion or reciprocal recombination with loss of one of the products. The models are as follows.

(i) Recombination may initiate with a specific cleavage 5′ to the gene, but sequence transfer may begin various distances 3′ to the cleavage. In both the typical switch with complete or near-complete gene replacement and the incomplete switch described here, the 3′ recombination may have occurred in the same place, that is, at the downstream homology site. A precedent for this model is the mating type switch in *Saccharomyces cerevisiae*, in which a silent cassette containing a mating-type-specific Y region replaces another Y region at an expression site through unidirectional gene conversion (23). Usually, the recombining Y segments are heterologous. Recombination is initiated by a site-specific cleavage in the expression site immediately adjacent to Y; it usually terminates beyond Y in another region of flanking homology (36). However, when the silent cassette was substituted with a Y allele homologous to the expressed Y, conversion of expressed Y sequences occasionally began

not at the cleavage site but at a point within Y (24). Thus, initiation of recombination by a specific cleavage does not dictate that sequence replacement begin at the site of cleavage.

(ii) Recombination may be initiated by a cleavage at the telomere, near the DHS. A downstream cleavage in this location would meet the requirements for a unidirectional switch and provide a restricted site for initiation of recombination. The upstream recombination might then occur at any site bearing sufficient sequence similarity, including sites within the *vmp* genes themselves. It is possible that a nick at the telomere occurs in the process of plasmid replication. Proposed mechanisms for replication of linear hairpin DNA specificity involve nicks at the termini (14). As in the first model, transfer or duplication of sequence from the silent locus would begin at a site internal to the nick. Conservation of the DHS in cells of four other serotypes may be evidence of this region's importance in recombination (32).

The DNA rearrangements that produce antigen-specific antibodies in lymphocytes are parried by the DNA rearrangements that produce antigenic variants in relapsing fever *Borrelia* spp. The discovery of *vmp7/21* indicates that some switches are not complete, but the advantage for *B. hermsii* of a switching mechanism that permits partial gene switches is not known. The hybrid Vmp7/21 retained in Western blots reactivity to monoclonal antibodies directed against two serotypes, and presumably, on this basis, cells with this phenotype would be at a selective disadvantage in an immunocompetent host. However, it is possible that Western blot analysis underestimates the novelty produced by partial switches and chimeric Vmp's. Protective antibodies in mouse infections with *B. hermsii* often are directed against conformational epitopes (16a). Such epitopes may be substantially altered through creation of a hybrid protein, even though most or all of the linear epitopes in the two components of the chimera remain unaffected. Aforementioned finding of Stoenner of a "7 × 16" relapse population in mice infected originally with serotype 7 suggests that partial gene switches can yield cells that can escape the predominant antibody response in immunocompetent mice. Consideration of a similar phenomenon in trypanosomes suggests other benefits of partial gene switching.

African trypanosomes switch antigens by a mechanism similar to that of *B. hermsii* (16), and antigen switching is occasionally effected by partial gene conversions in these organisms also (30, 38). The chimeric genes created by these events often are retained, either because they undergo reciprocal recombination with silent genes or because the succeeding expressed antigen genes are activated in situ rather than by transposition (19). Chimeric genes so retained expand and evolve the trypanosome's antigen repertoire (30, 33). These chimeric antigen genes are often formed from pseudogenes (30, 33, 37, 38). Because pseudogenes lack complete open reading frames, partial gene replacement is required for their expression. Finally, some chimeras are formed from silent antigen genes that lack 5′ or 3′ homology blocks, the usual sites for recombination (38). Regions of homology within these genes serve as recombination sites. Partial gene replacement is thus necessary for expression of these genes as well. A switching mechanism that allows for variable recombination sites may have any or all of these functions in *B. hermsii* and other relapsing fever *Borrelia* spp.

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