Residual Brain Infection in Relapsing-Fever Borreliosis

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Background. Neurological involvement is common in the spirochetal infection relapsing fever (RF) in both humans and experimental animals. RF is best known for antigenic variation caused by the sequential expression of variable outer membrane lipoproteins of 2 sizes, variable small (Vsp) and variable large (Vlp) proteins. Less understood is the persistence of RF borreliae in the brain after they are cleared from the blood, referred to as residual brain infection (RBI). Our goal was to investigate the phenomenon of RBI in RF.

Methods. We studied RBI in immunocompetent mice by culturing blood and perfused brain samples 1 month after intraperitoneal inoculation with Borrelia turicatae serotype 1 (Bt1). Mice deficient in Toll-like receptor 2 (TLR2−/−) or in B and T cells (scid) were included for comparison.

Results. All scid mice had persistent infection in blood and brain. RBI was found in 3 (19%) of 16 immunocompetent and TLR2−/− mice. RBI was caused by either persistence of the original serotype (Bt1) or newly emerged Vsp (n = 1, renamed Bt3) or Vlp serotypes. The Vsp of Bt1 (Vsp1) and Bt3 (Vsp3) were 75% identical.

Conclusions. RBI in RF is relatively frequent and can occur by persistence of the original or newly emerged serotypes.

Brain infection is a characteristic feature of many pathogenic spirochetes, notably Treponema pallidium and the several Borrelia species that cause Lyme disease (LD) and relapsing fever (RF). The neurological complications of Borrelia infections are collectively referred to as neuroborreliosis [1, 2]. The most common manifestations of neuroborreliosis in RF are meningitis, facial nerve palsy, radiculitis, and encephalopathy (reviewed in [3]). In outbreaks of tickborne RF, neuroborreliosis can be the dominant clinical feature [4, 5]. Our laboratory has been studying the pathogenesis of neuroborreliosis in animal models using nonhuman primates infected with LD borreliae and mice infected with RF borreliae [6–8]. RF borreliae are best known for the phenomenon of antigenic variation, which allows them to spontaneously change their serotype by switching the expression of variable major proteins (VMPs) [9, 10] of 2 sizes, large (Vlp) and small (Vsp). A switch in VMP allows RF borreliae to escape killing by the host’s serotype-specific antibody response.

Less known is the phenomenon of residual brain infection (RBI), which refers to the tendency of RF borreliae to persist in the brain after they disappear from the blood (reviewed in [1]). The goal of the present study was to investigate the phenomenon of RBI in RF. The results revealed that RBI is relatively frequent in both immunocompetent and Toll-like receptor 2−/− mice. RBI can be caused by the persistence of spirochetes in the brain, after their clearance from the blood, of either the original infecting serotype or new serotypes that spontaneously emerge during infection.

MATERIALS AND METHODS

Strains and culture conditions. B. turicatae serotypes 1 (Bt1; formerly serotype A) and 2 (Bt2; formerly serotype B) have been described elsewhere [7, 11–13]. Borreliae were cultured in BSK II medium [14] and counted in a Petroff-Hauser chamber [10]. When mouse tissue samples were cultured, 50 μg/mL rifampin and 100 μg/mL phosphomycin were present in the medium. Plas-
ma samples from infected mice were used to start broth cultures, which were collected by centrifugation at 9000 g at cell densities of $1 \times 10^9$ cells/mL. The purity of Bt1 was assessed before inoculation by Western blot with anti-Vsp1 polyclonal antiserum [11, 12].

**Mouse infections.** Female mice, 4–6 weeks old, of different genetic backgrounds, purchased from Jackson or Charles Rivers or bred in-house (C57BL/6 TLR2$^{	ext{−/−}}$), were inoculated intraperitoneally with $1 \times 10^7$ Bt1 spirochetes in 300 μL of PBS. Groups of 4–8 mice each were used for all experiments. Mice sham-inoculated with PBS were used as controls. The housing and care were in accordance with the Animal Welfare Act. Infection was confirmed by mixing tail-vein blood with an equal concentration and isotype, was used as a negative control. Image analysis was done by scoring 5 × 100 microscopic fields per sagittal brain section as follows: 0, no F4/80-positive cells; 1, 1–2 cells/field; 2, 3–10 cells/field; 3, >10 cells/field; and 4, diffusely positive. The microscopic fields were from leptomeninges or brain parenchyma, and the examiner was blinded to infection status and genotype.

**Tissue and fluid collection.** Mice were anesthetized with isoflurane for euthanasia and necropsied as described elsewhere [6]. Total body perfusion with 30 mL of PBS was performed, to minimize blood contamination of the brain. Whole brains were homogenized in BSK II medium and centrifuged for 3 s at 7000 g; then, the supernatant inoculated into 6-mL BSK II culture tubes. Any brain homogenates in which blood contamination was detectable were discarded. All cultures from brain and blood were examined for spirochetes by phase-contrast microscopy for a 2-week period.

**Histopathological studies.** Brains were removed at necropsy, fixed in 4% paraformaldehyde for 48 h at 4°C, and embedded in paraffin. Hematoxylin-eosin (HE) staining was prepared using standard techniques. All tissue sections were examined with standard light microscopy by a trained neuropathologist (D.C.) blinded to the infection status. Inflammation was defined in accordance with characteristic morphological changes after HE staining. A 3-step streptavidin-peroxidase technique was used for immunohistochemical analysis, as described elsewhere [15, 16]. Paraformaldehyde-fixed sections were treated with 0.5 mg/mL protease type VIII (Sigma P-5380) for 10 min for antigen retrieval. Commercially available rat monoclonal antibody antimouse macrophage F4/80 at a 1:1000 dilution (Serotec) was used for the identification of activated macrophages/microglia. The chromogen was 3,3′-diaminobenzidine tetrahydrochloride. Tissue sections from uninfected mice were used as negative controls. Purified rat IgG of the same species (Sigma), matched for concentration and isotype, was used as a negative control. Image analysis was done by scoring 5 × 100 microscopic fields per sagittal brain section as follows: 0, no F4/80-positive cells; 1, 1–2 cells/field; 2, 3–10 cells/field; 3, >10 cells/field; and 4, diffusely positive. The microscopic fields were from leptomeninges or brain parenchyma, and the examiner was blinded to infection status and genotype.

**Protein analysis.** Whole cells from harvested cultures were subjected to SDS-PAGE analysis with 12.5% acrylamide [17]. For Western-blot analysis, proteins were transferred to nitrocellulose membranes (Millipore) that were then blocked with 3% (wt/vol) dried nonfat milk in 10 mmol/L Tris (pH 7.4) and 150 mmol/L NaCl (milk/TS) for 4 h [18]. After the membranes were washed 3 times with 0.3% milk/TS, they were incubated with anti-Vsp1 polyclonal antiserum diluted 1:50,000 in 0.3% milk/TS [15]. Alkaline phosphatase–conjugated anti rabbit antibody (Sigma) served as the second ligand. The blots were developed with nitroblue tetrazolium chloride/5-bromo 4-chloro 3-indolyphosphatase p-toluidine salt as the substrate (Pierce).

**DNA methods.** Plasmid-rich Borrelia DNA was prepared using the diethylpyrocarbonate method [6]. All expressed vsp

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**Table 1. Susceptibility to neuroborreliosis in mice of different genetic background inoculated with serotype 1 of *Borrelia turicatae.***

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Blood</th>
<th>Brain</th>
<th>Serotype in brain$^a$</th>
<th>Vestibular dysfunction$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swiss Websters (outbred)</td>
<td>0/4</td>
<td>1/4</td>
<td>Novel</td>
<td>1/4</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>0/4</td>
<td>1/4</td>
<td>Serotype 1</td>
<td>0/4</td>
</tr>
<tr>
<td>SWR/J</td>
<td>1/4</td>
<td>0/4</td>
<td>...</td>
<td>0/4</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0/4</td>
<td>1/4</td>
<td>Novel</td>
<td>0/4</td>
</tr>
<tr>
<td>C57BL/6 TLR2$^{	ext{−/−}}$</td>
<td>4/16</td>
<td>3/16</td>
<td>All novel</td>
<td>0/16</td>
</tr>
<tr>
<td>C57BL/6/scid</td>
<td>4/4</td>
<td>4/4</td>
<td>Serotype 1 in all</td>
<td>4/4</td>
</tr>
<tr>
<td>C3H/HeJ/scid</td>
<td>4/4</td>
<td>4/4</td>
<td>Serotype 1 in all</td>
<td>4/4</td>
</tr>
<tr>
<td>CB17/scid</td>
<td>4/4</td>
<td>4/4</td>
<td>Serotype 1 in all</td>
<td>4/4</td>
</tr>
</tbody>
</table>

$^a$ By Western blot with anti–variable small protein 1 polyclonal antibody.

$^b$ Spinning in circles when lifted off the ground by the tail.
genes were amplified and sequenced using a forward primer for the B. turicatae vsp promoter (5'-TTGAAAAATCGAAATTGTGAAATA-3', nt 9–32 on GenBank U85413) and a reverse degenerated primer for a conserved region on the 3' end of all known B. hermsii and B. turicatae vsp genes (5'-TGWAAGCK-SWKYDATTRECAG-3', nt 683–654 on GenBank U854130) [11, 12]. When it is annealed at 42°C, this primer pair specifically amplifies expressed vsp and vlp genes from B. hermsii (Bh7 and Bh21) and B. turicatae (Bt1 and Bt2). Because only genes placed in front of the promoter are expressed, using a promoter specific primer allows the study of gene expression by polymerase chain reaction (PCR) instead of reverse-transcription PCR. DNA amplification was performed in a thermal cycler using 50-μL reaction volumes that contained 1.25 U of Thermus aquaticus DNA polymerase (Roche), 25 pmol of each primer, 200 μmol/L each dNTP, 10 mmol/L Tris (pH 8.3), 50 mmol/L KCl, and 4 mmol/L MgCl2. The program was 40 cycles for 1 min at 94°C, for 1 min at 42°C, and for 2 min at 72°C, followed by a final extension cycle for 7 min at 72°C. Tubes with buffers and reagents but without DNA were included as negative controls for cross-contamination. DNA sequencing was performed 3 times on each strand using the cycle-sequencing method through commercial vendors (Retrogen). vsp3 was compared with previously characterized genes using WU-Blast2 software (version 2.0; Washington University). Vsp3 was compared to Vsp1 and Vsp2 using CLUSTAL W (version 1.82) multiple sequence alignment (available at: http://www.ebi.ac.uk/clustalw).

Statistical analysis. The 2-tailed Fisher’s exact test was used to compare differences in percentages, and nonparametric tests were used to compare differences between means. \( P < .05 \) was considered to be significant.

Table 2. Microglial/macrophage activation in brains of C57BL/6 wild-type or Toll-like receptor (TLR) 2–deficient (TLR2<sup>−/−</sup>) mice 1 month after inoculation of serotype 1 of Borrelia turicatae (Bt1) or PBS (as a control).

<table>
<thead>
<tr>
<th>Mouse strain, inoculation</th>
<th>Brain parenchyma</th>
<th>Leptomeninges</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bt1</td>
<td>0.75 (0–2.2)</td>
<td>7.5 (2.6–12.4)</td>
</tr>
<tr>
<td>PBS</td>
<td>0.75 (0–1.7)</td>
<td></td>
</tr>
<tr>
<td>C57BL/6 TLR2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bt1</td>
<td>0</td>
<td>10.3 (4.8–15.9)</td>
</tr>
<tr>
<td>PBS</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** Macrophages and microglia were detected by immunostaining with rat anti-mouse F4/80 monoclonal antibody. Results are given as mean (95% confidence interval) sum score for 5 × 100 microscopic fields. Scoring was done by an examiner who was blinded to infection status. The scoring system was as follows: 0, no F4/80-positive cells; 1, 1–2 cells/field; 2, 3–10 cells/field; 3, >10 cells/field; and 4, diffusely positive.

RESULTS

RBI in immunocompetent mice. To study the susceptibility to RBI of immunocompetent mice, we examined outbred and inbred mice of different genetic backgrounds. All mice were inoculated intraperitoneally with 1 × 10<sup>7</sup> B1 spirochetes, and the infection of blood and perfused brain was examined 1 month later by culture followed by SDS-PAGE and Western-blot analyses. The inbred strains were chosen arbitrarily on the basis of their known susceptibility to Lyme arthritis, another spirochetal disease. The strains chosen were C57BL/6 (H2b haplotype), which is moderately susceptible to Lyme arthritis [19], and C3H/HeJ (H2k) and SWR/J (H2q), which are highly susceptible [20]. Swiss Webster outbred mice were also included for the examination of RBI in a genetically heterogeneous population. RBI was defined as a positive brain culture in a mouse whose necropsy blood sample was culture negative. The results (table 1) showed that RBI was found in 3 (19%) of 16 immunocompetent mice. Although none of the inbred mice developed any signs of neurological dysfunction, 1 of 4 outbred mice developed severe head tilt, walking in circles, and spinning in the air when lifted off the ground by the tail. These manifestations of vestibular dysfunction were indistinguishable in time of onset and severity from those observed in scid mice (see below). Only 1 (6%) of 16 immunocompetent mice had positive blood cultures 1 month after inoculation (table 1). These results showed that RBI is relatively common in immunocompetent mice across different genetic backgrounds. Furthermore, they showed that, occasionally, immunocompetent mice develop vestibular dysfunction as a complication of RF borreliosis.

Figure 1. Assay in 12.5% SDS-PAGE of brain (br) and blood (bl) culture lysates from 4 [1–4] C57BL/6-scid mice inoculated with serotype 1 of Borrelia turicatae (Bt1) and necropsied 1 month later. Molecular-weight markers are shown in kilodaltons on the left, and the whole–serotype 1 spirochete lysate is shown on the right (Bt1), for comparison. The arrow points to 23-kDa variable small protein 1, the serotype-defining major outer membrane lipoprotein of Bt1.  

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Residual brain infection in immunocompetent mice.

Figure 2. Residual brain infection in immunocompetent mice. A, SDS-PAGE and B, Western blot with anti–variable small protein (Vsp1) polyclonal antibody of blood (lane 1) and brain (lanes 2–4) BSK II culture lysates from 4 immunocompetent mice (lanes 1–4) of different genetic backgrounds persistently infected with Borrelia turicatae (table 1). Molecular-weight markers are shown in kilodaltons on the left, and a lysate from serotype 1 (Bt1) is shown in lane 5 for comparison. B. Reactions only with the anti-Vsp1 antibody (lanes 3 and 5). The lysate from B. turicatae serotype 2 spirochetes did not react with the anti-Vsp1 antibody (data not shown). The arrow points to the 23-kDa Vsp1 in the Bt1-positive control.

RBI in TLR2−/− mice. The main virulence determinants of RF [21, 22] and LD [23] borreliae are lipoproteins. Because TLR2 is an important signal-transducing receptor for bacterial lipoproteins [24, 25], it is possible that TLR2 deficiency, by impairing recognition of the infection, might increase susceptibility to RBI in RF. To investigate this possibility, we cultured blood and perfused brain samples from TLR2−/− mice 1 month after inoculation with Bt1. The results revealed RBI in 3 (19%) of 16 TLR2−/− mice examined, similar to the frequency of persistent infection in blood samples (4/16 [25%]; table 1). Although the differences in persistent blood infection between immunocompetent (1/16 [6%]) and TLR2−/− (4/16 [25%]) mice did not reach statistical significance, we cannot rule out a type II error, because the number of mice used was not large. It is also possible that TLR2 deficiency may influence the spirochetal load in other tissues that we did not study. None of the TLR2−/− mice developed any signs of vestibular dysfunction.

Because TLR2 plays an important role in the inflammatory response to LD borreliosis [25], we studied whether TLR2 deficiency had any effect in the inflammatory response to the infection in the brain of mice with RF borreliosis. For this, the brain from C57BL/6 wild-type and TLR2−/− mice necropsied 1 month after intraperitoneal inoculation with Bt1 were examined microscopically. The examination of HE-stained slides showed minimal to mild meningitis in both groups (not shown). Immunohistochemical staining with an antibody to the glycoprotein F4/80, a marker of mouse microglia/macrophage activation [26], revealed increased signals, mainly in the leptomeninges. Image analysis of F4/80-immunostained sections revealed that the extent of macrophage/microglia activation was similar in wild-type and TLR2−/− mice (table 2). These results revealed that TLR2 deficiency does not increase susceptibility to RBI or brain inflammation in RF borreliosis. Other factors can compensate for the lack of TLR2.

RF neuroborreliosis in scid mice. Previous studies showed that CB17-scid mice are very susceptible to RF borreliosis, including brain infection, when they are inoculated with Bt1 [7, 16]. To investigate whether the susceptibility to brain infection in scid mice is influenced by other background genes, we compared neuroborreliosis in scid mice of 3 different genetic backgrounds—CB17, C3H/HeJ, and C57BL/6—after inoculation with Bt1. In contrast to immunocompetent and TLR2−/− mice, all scid mice developed persistent infection of the blood and brain and vestibular dysfunction, independently of their genetic background (table 1). SDS-PAGE analysis of positive blood and brain cultures from all scid mice showed the presence of a single VMP band of 23 kDa (figure 1). As expected, Western-blot analysis with anti-Vsp1 polyclonal antiserum confirmed that the band was Vsp1 (not shown). These results showed that the scid immunodeficiency significantly increases the frequency of blood and brain infection, compared with that in immunocompetent and TLR2−/− mice, from just under 20% to 100% (P < .0001). Similarly, all scid mice developed clinical manifestations of vestibular dysfunction.

Analysis of B. turicatae serotypes causing RBI. In contrast to the finding in scid mice, which all had a single VMP band of 23 kDa (Vsp1), SDS-PAGE analysis of the 3 serotypes causing RBI in the immunocompetent mice showed that only 1 still had a 23-kDa VMP band (figure 2, lane 1). The other 2 (figure 2, lanes 2 and 4) had much larger VMP bands, corresponding to members of the Vlp families [27]. Similarly, the one serotype causing persistent blood infection in immunocompetent mice...
Figure 4. Comparison of deduced protein sequences of variable small protein (Vsp) 1, Vsp2, and Vsp3 of Borrelia turicatae. CLUSTAL W (version 1.82) multiple sequence alignment (available at: http://www.ebi.ac.uk/cgi-bin/clustalw/) showed that Vsp3 is very similar to Vsp1 and Vsp2 in the proximal and distal thirds, whereas the middle third is more heterogeneous. In the consensus line, an asterisk indicates strict conservation, meaning that the same residue is found in the considered position in compared amino acid sequences; a colon indicates conserved substitutions, meaning that chemically similar residues are found in the considered position in compared amino acid sequences (e.g., all basic residues [R or K], or all acidic residues [D or E]); and a period indicates semiconserved substitutions, meaning that residues found in the considered position are more variable than in the conserved case but are still considered to be chemically similar (e.g., all polar residues [R, K, D, E, S, or T]).

The analysis by SDS-PAGE of the 3 relapse serotypes causing RBI in TLR2−/− mice (figure 3A, lanes 2–4) showed a similar situation: only 1 of the lanes (lane 3) had a VMP of 23 kDa, identical to that of Vsp1 (lane 1). The other 2 (lanes 2 and 4) had VMP bands of ≥37 kDa, a typical molecular size of members of the 5 Vlp families [28]. In contrast to immunocompetent mice, the 23-kDa VMP band in TLR2−/− mice (figure 3B, lane 3) was not recognized by anti-Vsp1 polyclonal antiserum. The implication of this finding is that a new member of the Vsp family of B. turicatae was responsible for RBI in 1 of the TLR2−/− mice. This relapse serotype was cloned by limiting dilution in BSK II media [29], renamed serotype 3, and further characterized.

Characterization of B. turicatae serotype 3. Serotype 3 spirochetes were found to be infectious for newly inoculated CB17-scid mice and were confirmed to be neurotropic by infecting the brain of all 3 scid mice examined (not shown). A PCR product of the predicted size was amplified from serotype 3 plasmid DNA using a forward primer specific for the B. turicatae vsp1 and vsp2 promoter region [11, 12] and a degenerated reverse primer based on a conserved sequence at the 3′ end of all known vsp genes from B. hermsii and B. turicatae [27]. The deduced amino acid sequence (figure 4) revealed that Vsp3 is a member of the Vsp/OspC family of Borrelia proteins [30]. The proximal and distal thirds were highly conserved, whereas the middle third was hypervariable (figure 4). Analysis of the DNA sequence homology revealed the highest homology of vsp3 was with B. turicatae vsp1 (75%; table 3). vsp3 was 64%–74% similar to vmp genes from other RF borreliae and 62%–66% similar to ospC genes from LD borreliae (table 3).

DISCUSSION

The major findings of the present study were as follows. (1) RBI occurs in close to 20% of immunocompetent mice inoculated with a neurotropic strain of B. turicatae. (2) TLR2 deficiency does not increase susceptibility to RBI. (3) The scid mutation confers 100% susceptibility to RF neuroborreliosis, independently of background genes. (4) Vestibular dysfunction is the main clinical manifestation of RF neuroborreliosis in mice. (5) RBI can be caused by persistence of the original infecting serotype or by newly emerged serotypes that spontaneously arise in the population.

Interest in the phenomenon of neurological involvement by Borrelia species dates to the early twentieth century, when neurological complications were frequent in patients with RF; however, research was almost abandoned after effective treatment with penicillin became available (reviewed in [1]). Earlier investigators in the field were especially interested in the ability of some RF spirochetes to persist in the brain for a long time after they disappear from the blood, referred to as RBI [31, 32].
RBI was the subject of many investigations during the last century [1]. It was shown to occur not only in experimental animals but also in nature [33, 34]. RBI was documented in experimental animals for up to 3 years after inoculation [35]. Borreliae causing RBI are susceptible to the serum from the animal from which they are recovered, and they cannot reenter the blood from the brain [36]. In contrast to RF, RBI has not been documented in animal models of Lyme borreliosis [37–39]. In a recent study, we did not find a single case of RBI in Balb/c mice 1 month after inoculation with several different strains of LD borreliae, most of which were isolated from human cerebrospinal fluid [40]. In contrast, brain involvement is a frequent occurrence in many species of RF borreliae [1, 6, 7, 41, 42]. A major recent advance in the field of RF has been the elucidation by Barbour et al. [43–45] that a switch in the variable major protein (VMP) family of relapsing-fever borrelia genes has been changed from the original vmp to vlp (variable large protein) genes, and letters have been replaced by nos. For example, Vsp1 was initially referred to as VmpA [11], then VspA [49].

Table 3. Genes most closely related to *Borrelia turicatae* vsp3.

<table>
<thead>
<tr>
<th>Borrelia species</th>
<th>Disease</th>
<th>Genes</th>
<th>Similarity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. turicatae</em></td>
<td>Relapsing fever in the US</td>
<td>vsp&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vsp2, vsp4, vsp5, vsp6</td>
<td>74</td>
</tr>
<tr>
<td><em>B. hermsii</em></td>
<td>Relapsing fever in the US</td>
<td>vsp3, vlp7, vlp21</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vsp3, vsp22, vsp13, vsp26</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vsp24, vsp6, vsp17, vsp25</td>
<td>66</td>
</tr>
<tr>
<td><em>B. duttoni</em></td>
<td>Relapsing fever in Africa</td>
<td>vmp (unnamed)</td>
<td>66</td>
</tr>
<tr>
<td><em>B. valaisiana</em></td>
<td>Lyme disease–like species from Eurasia&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ospC</td>
<td>66</td>
</tr>
<tr>
<td><em>B. afzelii</em></td>
<td>Lyme disease in Europe</td>
<td>ospC</td>
<td>65</td>
</tr>
<tr>
<td><em>B. miyamotoi</em></td>
<td>Relapsing fever–like species from Japan&lt;sup&gt;c&lt;/sup&gt;</td>
<td>vmp (unnamed)</td>
<td>64</td>
</tr>
<tr>
<td><em>B. burgdorferi sensu stricto</em></td>
<td>Lyme disease in the Western Hemisphere</td>
<td>ospC</td>
<td>65</td>
</tr>
<tr>
<td><em>B. garinii</em></td>
<td>Lyme disease in Europe</td>
<td>ospC</td>
<td>65</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined using the Washington University Basic Local Alignment Search Tool (version 2.0).

<sup>b</sup> The nomenclature for the variable major protein family of relapsing-fever borrelia genes has been changed from the original vmp to vsp (variable small protein) or vlp (variable large protein) genes, and letters have been replaced by nos. For example, Vsp1 was initially referred to as VmpA [11], then VspA [49].

<sup>c</sup> These species have been isolated from ticks and rodents, but no human infections have been reported.

susceptible to neuroborreliosis after inoculation with Bt1 (D.C. and H. Gelderblom, data not shown). In contrast to the scid mutation, we did not find any evidence that TLR2 deficiency increases susceptibility to RF neuroborreliosis: the frequency of RBI, cerebral microgliosis, and vestibular dysfunction was similar in wild-type and TLR2-deficient mice. This is in contrast to the case in Lyme borreliosis, in which TLR2 deficiency increases the severity of arthritis and the spirochetal load in some tissues [25].

Previous studies of *scid* mice inoculated with *B. turicatae* [7, 15] and Balb/c mice inoculated with *Borrelia hermsii* [6] showed significant differences in the ability of the individual serotypes to enter the brain. Because the only difference between isogenic *Borrelia* serotypes is their VMPs, this suggests that expressing certain VMPs (like Vsp1) may facilitate brain invasion. In other words, some RF VMPs may function as spirochetal “neuroinvads.” This suggests that another consequence of VMP variation in RF may be a gain or loss of function with regard to brain infection. Sequence analysis of Vsp3 showed that it is closely related to Vsp1 (table 3 and figure 4). This suggests that a VMP switch from Vsp1 to Vsp3 may have allowed *B. turicatae* to escape killing while still preserving its ability to infect the brain. Sequence analysis of *B. turicatae* Vsp1, 2, and 3 showed they are very similar in the proximal and distal regions, with most of the variability concentrated in the middle third (figure 4). Localized polymorphisms in this middle region of Vsp [49] may account for differences of individual *Borrelia* serotypes in the ability to infect the brain.

In previous studies of Balb/c mice inoculated with *B. hermsii* serotype 7, we found that it had been cleared not only from the blood but also from the brain by the time of the first relapse [6]. In this study, we also found that an established serotype 7 brain infection was cleared from the brain of irradiated Balb/
c mice by the intravenous administration of anti-Vsp7 monoclonal antibodies of the IgG but not of the IgM isotype [6]. This demonstrated that some antibodies are better than others at clearing spirochetes from the brain. However, we have now found Bt1 in the brain of an immunocompetent mouse that had been inoculated with Bt1 1 month earlier. This is, to our knowledge, the first demonstration that the original infecting serotype can somehow escape the host’s antibody response and survive in the brain. The implication is that, occasionally, immunocompetent hosts fail to clear spirochetes from the brain, resulting in RBI. The mechanism that allowed Bt1 to survive in the brain but not in the blood remains to be determined. Because the spirochetes cultured from the brain were expressing Vsp1, it is more likely that the antibody response of this particular C3H/HeJ mouse had difficulty reaching or killing the spirochetes that had entered the brain. Although the number of mice was small, it was also interesting that one of the outbred mice developed vestibular dysfunction as severe as that of scid mice. This also implies that some hosts, who apparently are immunocompetent, fail to control RF borreliosis, resulting in neurological complications. Contrary to the case in humans, in whom neuroborreliosis involves predominantly the seventh cranial nerve both in RF and LD [3], in mice, RF neuroborreliosis predominantly affects the vestibular system [7, 15].

The present results reveal that persistent brain infection in RF borreliosis is not only a feature of antibody-deficient mice but that it also occurs in a significant percentage of immunocompetent mice. RBI can be caused by the persistence in the brain of the original or relapse serotypes after they had been eliminated from the blood. Future studies may elucidate how variations in VMPs modulate the ability of RF borreliae to enter the brain.

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References

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