Antibody Responses Elicited against the *Treponema pallidum* Repeat Proteins Differ during Infection with Different Isolates of *Treponema pallidum* subsp. *pallidum*

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Variation in the expression of the different Tpr proteins in the syphilis spirochete, *Treponema pallidum* subsp. *pallidum*, may have important implications in its ability to evade host immune detection and cause persistent infection. In the present study we examined the pattern of antibody responsiveness to different Tpr members during infection with three isolates of *T. pallidum*. There was variability in the specificities and temporal patterns of reactivity of the antibodies elicited against the individual Tpr proteins, suggesting that isolates may express different repertoires of Tpr proteins during infection.

Syphilis is a chronic disease caused by *Treponema pallidum* subsp. *pallidum*. During the early stages of infection, the host mounts a vigorous immune response that is able to clear the majority of the treponemes from early lesions but is unable to completely eradicate the infection. Phagocytosis of opsonized treponemes by macrophages is the primary mechanism by which the host immune system clears treponemes from early lesions (1, 13, 15). While *T. pallidum* has been shown to be susceptible to bactericidal and opsonic antibodies (2-5, 15), the inability to culture the organism, combined with the fragile nature of its outer membrane, have made the positive identification of outer surface molecules that may be antibody targets difficult and often inconclusive (8).

The *T. pallidum* repeat (tpr) gene family is a 12-member gene family, originally identified in the Nichols strain of *T. pallidum*, that encodes proteins which share amino acid homology with the major surface proteins of *Treponema denticola* but whose function is unknown (5–7, 10). The 12 members of the gene family are divided into subfamily I (TprC, D, F, and I), subfamily II (TprE, G, and J), and subfamily III (TprA, B, H, K, and L) based on regions of sequence conservation shared by members within the same subfamily (5). TprK and members of subfamily I have putative cleavable signal peptides. Previous studies have shown that TprK is a target of opsonic antibody and that immunization with recombinant TprK is partially protective to challenge with *T. pallidum*, possibly suggesting a surface location for TprK (5, 16), although this conclusion is controversial (12). More recently it has also been shown that multiple alleles of tprK exist within isolates of *T. pallidum* subsp. *pallidum* (6), and there is some heterogeneity in tprD (7) and tprI (18; unpublished data) sequences among strains. The Chicago and Baltimore 73-1 (Bal 73-1) strains share the same tprC and tprD sequences as the Nichols strain. Preliminary examination of sequence variation in the tprE, F, G, and I genes among *T. pallidum* strains has revealed very minor heterogeneity relative to that observed with the tprD, J, and K genes (unpublished data). Information regarding the degree of sequence heterogeneity among strains in the tprA, B, H, and L genes is currently unavailable. A possible surface location for some tpr gene products and sequence variation in some tpr genes suggest that the Tpr family could contribute to immune evasion and persistence of *T. pallidum*. In the present study we sought to determine which of the Tpr members in two “street” isolates of *T. pallidum*, Chicago and Bal 73-1, are expressed during infection and are thus recognized by the immune system; this pattern of antibody responsiveness was compared to the antibody response elicited by the laboratory-adapted Nichols strain.

The Nichols strain of *T. pallidum* has been propagated in rabbits since its initial isolation from the cerebrospinal fluid of a patient with secondary syphilis in 1912 (17). Despite extensive propagation in rabbits, it remains pathogenic for humans as demonstrated by accidental laboratory infections (9, 19). The Bal 73-1 strain was isolated from a newborn child with congenital syphilis in 1969 (11), and the Chicago strain was isolated in 1951 from a primary chancre (19). The Bal 73-1 and Chicago strains were passed in rabbits within our lab three and nine times, respectively, prior to their use in these studies. We have no information concerning the number of passages for these strains prior to our obtaining the stocks (from Paul Hardy and Ellen Nell, John Hopkins University); however, this number is certainly much lower than for the Nichols strain.

Three to six separate animals per strain were infected intratesticularly with 10⁶ treponemes as previously described (14). Sera were collected from animals prior to infection and at days 10, 17, 30, 45, 60, and 90 postinfection (day 45 samples were not available from Nichols-infected animals). Enzyme-linked immunosorbent assays (ELISAs) were performed using recombinant peptides that were derived from the sequences of the tpr genes originally reported in the *T. pallidum* (Nichols) genome sequence and then produced in *Escherichia coli* (10). The peptides were histidine tagged and purified on nickel columns (5). TprC and D are identical in the Nichols strain, so

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followed by a 4°C overnight incubation. The wells were then washed five times and used at a 1:100 dilution. The appropriate Tpr antigen resuspended in phosphate-buffered saline (PBS) containing 0.1% sodium azide and 0.1% sodium dodecyl sulfate and used at a final concentration of 10 μg/ml at 50 μl/well. Plates were incubated at 37°C for 2 h, followed by a 4°C overnight incubation. The wells were then washed three times with PBS (used for this and all subsequent washing steps) and blocked by incubation for 1 h at room temperature with 200 μl of 3% nonfat milk–PBS/well. After washing the wells, 100 μl of the primary antibody solution (made from pools of infected or normal rabbit sera diluted 1:20 in 1% nonfat milk–PBS–0.05% Tween 20 [Sigma]) was added to each well, allowed to bind for 1 h at room temperature, and then washed three times. To reduce background due to the independent recognition of some antigens in a subfamily, demonstrates that the antibody reactivity measured in our assays is primarily directed to the variable regions of the recombinant peptides rather than to the small constant regions present on most of the peptides. However, reactivity against our TprF peptide, which contains a 262-aa stretch of the 5° constant region, may be directed either to the 5° constant region of subfamily I or to the TprF variable regions; this distinction could not be determined in our studies. Furthermore, TprF is identical to TprI except for a stretch of 11 unique amino acids within its variable region (5). Thus, the strong level of antibody reactivity observed in Chicago-infected animals against our TprF peptide may actually be induced by expression of any of the subfamily I proteins, all of which contain this conserved region (Fig. 1). Further studies are planned to determine which of the subfamily I proteins is recognized by the antibody in the sera of the rabbits tested.

**Table 1. Recombinant peptides**

<table>
<thead>
<tr>
<th>Subfamily and peptide</th>
<th>Amino acid sequence limits</th>
<th>Size (aa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subfamily I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TprC/D</td>
<td>RLTLPE............ YTHLLIT</td>
<td>200</td>
</tr>
<tr>
<td>TprF*</td>
<td>YAVGLT............ GTGGGAC</td>
<td>349</td>
</tr>
<tr>
<td>TprI</td>
<td>RLTLPE............ YTHLLIT</td>
<td>211</td>
</tr>
<tr>
<td>Subfamily II</td>
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<td></td>
</tr>
<tr>
<td>TprE</td>
<td>RLTLPE............ OQTVAAD</td>
<td>191</td>
</tr>
<tr>
<td>TprG</td>
<td>RLTLPE............ DLIPKT</td>
<td>219</td>
</tr>
<tr>
<td>TprJ</td>
<td>RLTLPE............ MRTEIT</td>
<td>226</td>
</tr>
<tr>
<td>Subfamily III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TprA*</td>
<td>MGLVVT............ GCKITW</td>
<td>368</td>
</tr>
<tr>
<td>TprB</td>
<td>RLTLSP............ SLSKLIV</td>
<td>194</td>
</tr>
<tr>
<td>TprH</td>
<td>RITLTPE............ YTHLID</td>
<td>180</td>
</tr>
<tr>
<td>TprK</td>
<td>IEGYAE............ LTSFLE</td>
<td>315</td>
</tr>
</tbody>
</table>

*The peptide for TprF contains 262 aa of the 5° constant region of subfamily I in addition to 87 aa of the variable region.

b TprA consists of two overlapping reading frames. Our peptide is encoded by the second reading frame.
under way to determine whether there are antibody responses specifically directed to the constant regions of the Tpr proteins. These results demonstrate that different specificities of antibody responses are induced by infection with individual isolates of *T. pallidum*, suggesting that *T. pallidum* strains may express different repertoires of Tpr proteins. This may reflect intrinsic strain-to-strain differences in expression, or it may reflect the fact that the Nichols strain is highly rabbit adapted.
with Tpr expression distinct from that in strains that have been passed less extensively in rabbits. A difference in the antibody responses to our Tpr peptides was also observed in a previous study using a limited number of human samples (unpublished results). Extrapolation of our findings to natural human infection suggests that humans infected with individual strains of *T. pallidum* may be exposed to varying Tpr repertoires, depending upon their infecting strain. This possibility may need to be considered when designing a Tpr-based diagnostic test or vaccine.

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REFERENCES


