The spirochete now known as *Treponema pallidum* subsp. *pallidum* was first discovered as the etiological agent of syphilis in 1905 (19) and has been under investigation since that time. *T. pallidum* cannot be cultured in vitro and is propagated by serial intratesticular passage in rabbits. Most laboratory studies have been conducted using the rabbit-passaged Nichols type strain of *T. pallidum*, which was isolated from the cerebrospinal fluid of a patient with secondary syphilis in 1912 (16). A case of accidental laboratory exposure showed that *T. pallidum* Nichols was still infectious to humans more than 60 years after its isolation (5); however, it is not known whether continuous passage in rabbits has resulted in significant changes in the organism. *T. pallidum* has been called a “stealth pathogen” because it has very few integral membrane proteins, and the identities of these proteins are unknown (17, 18, 26). Studies performed in our laboratory and by others (1, 21), and the publication of the *T. pallidum* Nichols strain genome (6), identified a polymorphic family of genes, the *tpr* genes, several of which are predicted to encode integral outer membrane proteins. The gene that encodes one of these putative outer membrane proteins, *tprK*, is found in a single expression locus in the *T. pallidum* chromosome (3, 20). *T. pallidum* isolates that have been isolated more recently and propagated fewer times in rabbits than the Nichols strain have been shown to have diverse *tprK* sequences in the expression locus (2, 3, 9). Sequence diversity in *tprK* is localized to seven discrete variable regions, and the individual V regions have different levels of diversity within a given isolate (3, 9). Antibodies are produced against the V regions of *TprK* (15), and slight changes to the amino acid sequence of a particular V region appear to abrogate antibody binding (14). Thus, *tprK* sequence diversity represents an antigenic variation system in *T. pallidum*.

Our laboratory has proposed a gene conversion model for the generation of *tprK* diversity (3). In addition to the expression locus for *tprK*, we have found sequence cassettes representing diverse whole or partial variable region donor sequences in areas of the *T. pallidum* chromosome flanking the 5' and 3' ends of another *tpr* gene, *tprD*. In this model, the donor cassettes are copied and inserted into the expression locus, creating new, diverse V region sequences (3). V regions and corresponding donor sequences have flanking and internal four-base repeats that may facilitate recombination. During diversification of the *tprK* V regions, the donor sites do not undergo any variation, supporting a gene conversion mechanism for diversification (3).

The Nichols strain of *T. pallidum* has been maintained in various laboratories for decades. In contrast to the heterogeneity observed in the *tprK* locus of other isolated *T. pallidum* strains (3) and *T. pallidum* samples examined directly from humans (9), the Nichols strain maintained at the University of Washington in Seattle (NicholsSea) contains a single *tprK* sequence (2). The Nichols strain now maintained at the University of Connecticut in Farmington and the Nichols strain used to sequence the genome (6) were, like ours, originally obtained from James Miller at the University of California at Los Angeles but have been propagated separately for at least the past 10 years.
years. These strains appear to have only minor differences among their tprK sequences (6, 7). The University of North Carolina Nichols strain was not obtained from the University of California at Los Angeles and is the only one with reportedly diverse tprK sequences (20). We hypothesized that the lack of heterogeneity seen in the NicholsSea strain, and potentially other Nichols strains, is due to a lower rate of accumulation of tprK sequence diversity than that in non-Nichols strains. In the studies described here, we show that new tprK sequences are identified in NicholsSea only when organisms are harvested during the resolution of initial infection by the adaptive immune response. This phenomenon differs greatly from that seen in clonal Chicago C, a T. pallidum strain isolated from a heterogeneous parent, which rapidly gains diversity in tprK even when harvested early during infection. These results point to the possibility that, in the last 92 years of propagation, significant adaptation to the rabbit host may have occurred in the Nichols strain.

MATERIALS AND METHODS

Propagation of treponemes. Treponemes were propagated by serial intratesticular passage (12) in seronegative New Zealand White rabbits (R&R Rabbity, Stanwood, Washington). For the Nichols strain, peak numbers of organisms are present at day 9 to 10 postinfection; bacterial clearance begins at day 13 when specific immunoglobulin G levels are increasing (10). Between 13 and 17 days, the majority of treponemes are cleared from the testes by the adaptive immune response, and by 30 days postinfection, relatively few bacteria remain in the tissues (11). In this study, the Nichols strain was propagated by rapid and slow passage, carrying out two lines of propagation of treponemes harvested initially from rabbit 6308 (the inoculum or “founder” organisms). To propagate organisms by slow passage, significant adaptation to the rabbit host may have occurred even when harvested early during infection. These results point to the possibility that, in the last 92 years of propagation, significant adaptation to the rabbit host may have occurred in the Nichols strain.

Quantitative PCR. For the quantification of treponemal DNA from harvests that were unable to be quantitated by microscopy (due to low numbers of bacteria), a LightCycler (Roche Diagnostics) real-time PCR assay using the T. pallidum TpN47 gene was employed. For this analysis, aliquots of treponemal suspensions were mixed with an equal volume of 2× DNA lysis buffer (20 mM Tris, pH 8; 0.2 M EDTA, pH 8; 1.0% sodium dodecyl sulfate), and DNA was extracted using the QIAamp DNA minikit (QIAGEN) as previously described (9). Primers 5’-CAAGTACGGGGAACACCTG and AGTGAATCCCG AGCCGA amplify a 175-bp fragment of the TpN47 gene. The PCR reaction mixture contained 2 µl of the 10× reaction mix (LightCycler FastStart Master SYBR Green I; Roche Diagnostics), 3 mM MgCl₂, 1 µM (each) primer, and 3 µl of template DNA (diluted 1:5) in a final reaction volume of 20 µl. Each sample was tested in triplicate. A positive control, plasmid DNA containing a portion of the TpN47 gene including the 175-bp fragment, and a negative control, PCR-grade water instead of template DNA, were included in the assay. Samples were amplified as follows: an initial denaturation step at 95°C for 10 min followed by 50 cycles of denaturation at 95°C for 10 seconds, primer annealing at 64°C for 7 seconds, and extension at 72°C for 7 seconds. After each cycle, the temperature was raised to 82°C and fluorescence of SYBR green bound to double-stranded DNA was measured at 530 nm (LightCycler fluorescence channel F1). The crossing point, or the cycle number at which the fluorescence of the sample exceeded that of the background, was determined by the LightCycler software (version 3.5) using the second derivative method. Crossing points of the samples were compared to external plasmid DNA standards to determine the quantity of T. pallidum DNA in each sample. To rule out the possibility of nonspecific or primer-dimer amplification, a melting-curve analysis was performed after the amplification phase. The passage and collection of the clonal Chicago C strain are described elsewhere (3). DNA from Nichols and Chicago C treponemes was extracted as previously described (9).

Amplification and sequence analysis of tprK. DNA from the Nichols strains maintained at different laboratories was provided by the Centers for Disease Control and Prevention in Atlanta, Georgia (NicholsCDC), and the University of Texas, Houston (NicholsHou). The tprK gene was PCR amplified from NicholsCDC and NicholsHou and from the Seattle Nichols (NicholsSea) passages described above, using tprK-flanking primers (sense, 5’-AGTAAGTTTGTTCGCACTG, and antisense, 5’-CCATACATCTCCCTACAACTG) (2). Cycling conditions were as follows: denaturation at 94°C for 3 min and then 45 cycles of 94°C for 1 min, 60°C for 2 min, and 72°C for 1 min, with a final elongation step of 72°C for 10 min. The tprK amplicon was ligated into the pCR-II TOPO vector (Invitrogen) and sequenced as previously described (2); five tprK sequences were determined for NicholsCDC and NicholsHou, and 10 sequences were determined for the NicholsSea inoculum and subsequent passages. TprK sequences were aligned using ClustalW (24), and alignments were adjusted manually to correct for misalignments introduced by the program.

Analysis of T. pallidum rec genes. Full-length recA, recF, recG, recE, and recN genes were amplified from DNA from the T. pallidum strains NicholsSea, NicholsHou, and NicholsCDC clones and NicholsHou clones are aligned with the published TprK V region consensus sequences from the University of Washington Nichols strain (NicholsSea), the University of Connecticut Nichols strain (NicholsFarm), and the T. pallidum genome (NicholsGen). All five of the NicholsHou sequences were different from each other in at least one V region. Four of the five NicholsCDC sequences obtained were identical and are shown as a single sequence [labeled NicholsCDC(4) in Fig. 1]. One NicholsCDC clone, NicholsCDC.11, has two isolated amino acid changes in V6, but these changes are not templated by the donor sites identified to date (3). The rare base changes that result in single amino acid changes in the V regions are likely a result of point mutation or error during PCR amplification; similar rates of single base changes are also seen in the conserved regions of tprK. In this report, we focus our attention on those sequence changes that are likely produced by gene conversion with donor sites (called “templated changes”). Templated changes are seen in several TprK V regions derived from the University of Texas (NicholsHou) strain (Fig. 1). Among the five clones that were sequenced from NicholsHou, three different sequences are found in V1 and V6 and two different sequences are seen in V3, V5, and V7. NicholsHou was the strain originally used to determine the T. pallidum genome sequence, and several base pairs in the variable regions V1, V5, and V6 have remained unresolved (http://www.stddgen.lanl.gov/stddgen/bacteria/t_pal/). This analysis of five tprK sequences from the NicholsHou strain shows that there is variability in those V regions, suggesting that NicholsHou was diverse in tprK at the time that its genome was sequenced and perhaps explaining why the sequences of some V regions were not resolved in the genome sequence (6). Further, a 3-amino-acid stretch in the V7 sequence published in the genome is different from the recent NicholsHou V7 sequences that were found in our analysis (Fig. 1), suggesting that the NicholsHou
strain underwent changes in its V7 sequence sometime after the sequencing of the *T. pallidum* genome.

Among the four Nichols strains, V2 and V4 are conserved, and V5 is nearly identical. NicholsCDC V1, V3, V6, and V7 sequences are identical to the published NicholsFarm sequence, and in a similar fashion, the NicholsSea sequences of these four V regions are found among the NicholsHou sequences. Such strong conservation in TprK V regions is not observed among the *T. pallidum* strains that have been passed less frequently (2, 9), suggesting that these Nichols strains accumulate a much lower degree of diversity in TprK with routine passage in rabbits. This is supported by the observation that TprK sequences obtained from NicholsSea in 1995 are virtually identical to the current NicholsSea sequence (data not shown).

Diversity of *tprK* accumulates in *T. pallidum* Nichols upon slow passage but not with rapid passage. The observations that some Nichols strains have diverse TprK sequences and that *tprK* sequences vary somewhat among Nichols strains propagated in separate laboratories suggest that this strain retains the ability to develop new TprK sequences. To test this hypothesis, organisms were serially passaged at the peak of infection (every 9 to 10 days, before immune clearance had occurred; rapid passage) for 22 passages. The *tprK* gene was sequenced from the inoculum (founder) and after 22 rapid passages (RP22); 10 sequences were determined, and the translated amino acid sequences for the V regions are shown (Fig. 2). These sequences are essentially identical to one another. With the exception of the Q-to-K change in the V5 region of the Founder.24 sequence, the single amino acid differences seen in the founder sequences are not templated by the donor sites. None of the differences seen after 22 rapid passages represent templated changes.

The generation time for *T. pallidum* Nichols during early infection has been estimated to be 30 to 33 h (4); thus, 10 days of growth represents approximately seven to eight replication
FIG. 2. V region amino acid sequences after routine serial (rapid) passage of Nichols at the peak of infection (in the absence of an adaptive immune response). The top alignment represents 10 sequences from the inoculum (founder), and the lower alignment shows 10 sequences from treponemes after 22 rapid passages (RP22). N- and C-terminal amino acids and number of amino acids conserved between V regions are the same as in Fig. 1. Scattered amino acid changes, such as the K-to-R change in V2 (RP22.4), are thought to represent point mutations or errors due to PCR amplification. Shading is as defined for Fig. 1.
cycles per passage. Very few sequence differences are observed after more than 150 replication cycles (22 rapid passages × 7 replication cycles per passage), possibly suggesting that the intrinsic rate of variation is low in this strain. Alternatively, the low level of sequence diversity seen after 22 rapid passages could be a result of positive selective pressure for these particular V region sequences in the rabbit host or the lack of negative selection (i.e., immune pressure) against these sequences.

To examine the effect of the increase in the number of replication cycles and exposure to the adaptive immune response on tprK sequence variation, we passaged the same infectious inoculum in rabbits every 30 to 35 days for eight passages (slow passage). In this setting, organisms are allowed more time per infection (estimated to be as many as 22 to 28 replications per slow passage) for sequence change to occur, and by 30 days, the majority of organisms have been cleared by the adaptive immune response. V1 and V7 sequences in NicholsSea before and after several slow passages are compared in Fig. 3. The majority founder sequence is shown in the top line (V1 in the left column and V7 in the right column). After one slow passage (SP1), no changes were observed in
either V region. V2 to V6 also do not exhibit significant sequence change after one round of slow passage (see Fig. S1 in the supplemental material). After the second slow passage (SP2, approximately 50 cumulative replication cycles), all 10 V1 and V7 sequences have changed. These new sequences are different from the inoculum but identical in all 10 clones sequenced. V5 and V6 undergo the same phenomenon, a complete switch from the founder sequence at SP2, while V2, V3, and V4 sequences remain identical to the founder (see Fig. S1 in the supplemental material).

For each slow passage rabbit except SP2, the infectious inoculum was at least $10^6$ organisms, a number that is easily quantifiable by dark-field microscopy (where 10 organisms in 10 fields represents $10^6$ organisms per ml). Treponemes were not detectable by microscopy in the treponemal suspension that was harvested from the SP1 rabbit, raising the possibility that the organisms had undergone a “bottleneck” between SP1 and SP2, thus selecting for an infrequent variant in the population and resulting in the dramatic switch observed in V1, V5, V6, and V7 after two slow passages. To address this possibility, we used quantitative PCR to determine the concentration of organisms in the treponemal suspension harvested from the SP1 rabbit. By this method, we determined that the inoculum used to infect the SP2 rabbit contained $1.8 \times 10^4$ T. pallidum organisms (data not shown). Therefore, it is very unlikely that a bottleneck is responsible for the changes observed after the second slow passage.

After SP5, SP7, and SP8, several additional V1 and V7 sequences are introduced, and in V1, some reversion to the founder sequence is observed as early as SP5. Like V1 and V7, V5 and V6 undergo sequence change after two rounds of slow passage (see Fig. S1 in the supplemental material). Change in V3 and V4 was seen after SP5, but significant sequence diversity was never present in V2 (see Fig. S1 in the supplemental material). Thus, tprK diversification was seen after an estimated 50 replication cycles during slow passage, compared to no accumulation of diversity after at least 150 replication cycles of rapid passage. These results indicate that T. pallidum Nichols is indeed capable of generating tprK sequence diversity, but this diversity accumulates in the population only when the organisms are exposed to the adaptive immune response to T. pallidum.

**Analysis of Nichols sequence changes.** Our laboratory has proposed a model for a gene conversion mechanism for sequence change in the tprK V regions (3), and we previously have described V region changes by identifying the respective donor sites in Chicago C, a clonal strain derived from the heterogeneous Chicago strain (3). As described above, after two rounds of slow passage, all 10 V1, V6, and V7 sequences in the Nichols strain are nearly identical to each other but different from the founder sequence (V1 and V7 shown in Fig. 3). With continued rounds of slow passage, the new sequence that represents the majority at SP2 undergoes further sequence change. As examples, we have identified the donor templates that explain the variation that occurs in V1, V6, and V7 from founder to SP2 (Fig. 4A) and then from the new majority sequence at SP2 to SP5 (Fig. 4B). Sequences are shown in pairs, where the top sequence is either the founder (Fig. 4A) or the SP2 (Fig. 4B) sequence. The bottom sequence for each pair represents the newly generated V region sequence. New V regions can be explained by gene conversion in which sequences found in the donor sites are inserted into the expression locus as described in the work of Centurion-Lara et al. (3). In some cases, more than one donor site is needed to confer the observed sequence change. The substitution of the founder sequence DS37 in V1 confers a single base change which creates the glycine-to-serine amino acid change in addition to adding three new amino acids. Unlike most of the single base changes found elsewhere in the V regions and in the constant regions of tprK, this single-site change is found in a donor site and is observed more than once in SP2 and at subsequent passage levels. Thus, this glycine-to-serine change is unlikely to be a point mutation. Further analysis of the 3’-tprD flanking region has led to the discovery of two additional donor sites, DS48 and DS49. DS48 is 72 bases long and is a donor site for V5. It is found between DS19 and DS20. DS49 is a 33-base-long donor site for V7 found between DS21 and DS22. Our analysis also revealed that some of the donor sites are slightly longer than those originally identified. The changes are listed in Table S1 in the supplemental material.

**Reversion to Nichols founder sequence occurs in some V regions.** After two rounds of slow passage, the variable regions V1, V5, V6, and V7 contained completely different sequences than the founder (Fig. 3; see also Fig. S1 in the supplemental material). By SP5, only 6 of 10 V3 sequences and 7 of 10 V4 sequences were different from the founder sequence, showing that the founder sequence was reinstated in the population upon repeated passage. V2 is the only V region that did not undergo significant sequence change during the eight rounds of slow passage. We quantitated the “founder score” at each slow passage level by dividing the number of founder sequences seen at each slow passage level by the total number of sequences determined (10 in every case). Thus, a founder score equal to 1 indicates that every sequence is identical to a sequence seen in the inoculum, while a founder score of 0 indicates that every sequence has changed compared to the inoculum. Figure 5 shows founder score versus slow passage number for each TprK V region. Several V regions exhibited sequence change and then partially reverted to founder sequence. At SP2, V1 (open diamonds) and V5 (open circles) had no sequence identical to the founder sequence, yet at SP5, V1 and V5 had regained some founder sequence, and by slow passage 8, 7 of 10 V1 sequences and 3 of 10 V5 sequences were identical to the founder sequence. V3 (filled squares) also experienced partial loss of founder sequence (4 of 10 were identical to founder), but this loss did not occur until SP5, and the proportion of treponemes with founder V3 rebounded to 8 of 10 by SP8 (Fig. 5).

V6 and V7 (open squares and open triangles, respectively) also completely lost their founder sequence by SP2, but in contrast to V1, V3, and V5, V6 and V7 did not revert to founder sequence at later slow passages (Fig. 5). Two V regions, V2 and V4, showed the smallest amount of change during passage. V2 (filled triangles) was the least diverse of the V regions, with 9 out of 10 sequences identical to the founder sequence at SP5 (Fig. 5). V4 (filled diamonds) exhibited a slight decrease in founder score with slow passage; by slow passage 7, only 6 of 10 V4 sequences were identical to founder. The nonfounder V4 sequences observed at SP7 and SP8 were identical to each other (data not shown). The observation that

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FIG. 4. Examples of generation of V1, V6, and V7 diversity by gene conversion from founder to SP2 (A) and SP2 to SP5 (B). The top lines of each pair show the founder (A) or SP2 (B) amino acid and DNA sequences; the bottom lines show the sequences that arise at SP2 (A) or SP5 (B). The DNA sequence to be replaced is blue, and the new sequence is red; only those amino acids that changed, were inserted, or were deleted are shown in the appropriate color. Underlined sequences represent 4-bp direct repeats. The donor site(s) that conferred the change is shown between each of the V region sequences.
several V regions tend to remain as or revert to the founder sequence suggests that these particular V region sequences may be optimal for growth of the Nichols strain in the rabbit testis.

A gene conversion mechanism can explain the reversion of variable regions to founder sequence. As stated above, V region sequences that correspond to the founder sequence of NicholsSea are found in the tprD flanking donor regions. Figure 6 shows examples of how the reversion to founder in V1 and V5 can be explained by insertion of sequences from the donor sites. Three different donor sites are required to recreate the founder V1 sequence. The V5 example demonstrates how a single base change can be templated by a donor site. As shown in Table S1 in the supplemental material, there are 49 donor sites in the tprD flanking region. In these studies, the Nichols strain uses only a small fraction (13 of 49) of potential donor sequences to produce the sequence changes that were observed. In contrast, our previous study (3) showed that the Chicago C strain uses approximately three times as many donor sequences to create new V region sequences and appears to “mix-and-match” many different donor sites to create many more hybrid V region sequences than are seen in Nichols. This suggests that there may be selective use of certain donor sites in the Nichols strain, which has been passaged in rabbits since 1912, implying a positive selection for V region sequences that are optimal for growth in the rabbit testis.

The degree of tprK diversity between rapid-passaged clonal strains is markedly different. Previously, we showed that the clonal Chicago C strain spontaneously developed diversity in tprK in the absence of the adaptive immune response (i.e., during routine passage at the peak of infection) (3). To compare the amount of tprK diversity seen in clonal Chicago C after eight rounds of routine serial passage (RP8) to that seen in Nichols after 22 rounds of rapid passage (RP22), we analyzed 10 sequences for each strain. Diverse sequences are seen in clonal Chicago C RP8 for every V region except V3, and the heterogeneous V2 and V6 sequences (Fig. 7) have more amino acid changes, insertions, and deletions than the other rapid-passaged Chicago C V regions. In contrast, the RP22 Nichols sequences (Fig. 7) show striking sequence conservation and, specifically, no templated changes compared to the founder sequence. This demonstrates that strains other than Nichols accumulate diverse sequences even when organisms are rapidly passaged in the host, while Nichols maintains its clonal character under these conditions.

DISCUSSION

The tprK gene has been shown to have diverse sequences within and among all non-Nichols T. pallidum strains examined thus far (2, 3, 9, 20), and diversity in tprK variable (V) regions accumulates during infection (3, 9). Because tprK is found in a single locus, a given T. pallidum infection is composed of sub-populations of organisms with diverse tprK sequences. Here, we show that tprK diversity accumulates slowly in the Nichols strain, which has been maintained by intratesticular rabbit passage since the early 1900s. Sequence diversity is seen only when organisms are passaged slowly in the host, a setting that exposes organisms to the adaptive immune response. In contrast, much more extensive and rapid diversification is seen in the less frequently passaged clonal Chicago C strain, even when organisms are passaged rapidly. It has been proposed by Hazlett et al. (7) that diversity in Nichols tprK is caused by evolutionary drift, but this work and the findings of Centurion-Lara et al.

![Figure 5](http://iai.asm.org/)

FIG. 5. Change in abundance of founder V region sequences after slow passage. The founder score is shown on the y axis; a founder score of 1 indicates that every sequence is identical to founder, while a founder score of 0 means that no sequence was identical to founder. The number of passages is shown on the x axis, and each V region is indicated by a different symbol.

![Figure 6](http://iai.asm.org/)

FIG. 6. Examples of reversion to founder V1 and V5 sequences mediated by the donor regions. Colors are as defined for Fig. 4.
show that accumulation of diverse TprK sequences in Nichols is highly unlikely to represent a genetic drift phenomenon. Recombination of donor sequences into the tprK expression site through gene conversion seems to be the primary mechanism for accumulation of tprK diversity. Although such a mechanism does not rule out postconversion mutations in the V regions, these probably play a minor role in the generation of diversity.

The mechanism responsible for reduced variability in Nichols tprK is unclear. The T. pallidum genome sequence showed that, compared to other bacteria, many metabolic genes are missing from the Nichols strain (6), and a comparison with the related spirochete Borrelia burgdorferi demonstrates that, while the T. pallidum chromosome carries the recA gene, other common bacterial DNA repair genes are missing (22). The recA gene mediates antigenic variation of pilin subunits in Neisseria gonorrhoeae (8) and may be involved in antigenic variation in other bacteria, including T. pallidum. One might speculate that sequence differences between Nichols recA and those of other strains may change the function of Nichols RecA, resulting in the reduced rate of Nichols tprK diversity. To examine this, we compared the recA sequence from the T. pallidum Nichols genome (NicholsGen) with recA sequences from our Nichols strain (NicholsSea) and two heterogeneous strains, Chicago and Sea 81-4. We found no sequence differences among them except for two nonsynonymous nucleotide changes located outside of recognized functional domains in Sea 81-4 (see Fig. S2 in the supplemental material). Moreover, sequence differences were not observed between recF and recR from the NicholsGen and Chicago strains (see Fig. S3 in the supplemental material), and analysis of partial recG, recJ, and recN sequences has shown that these sequences are conserved between the two strains (data not shown). These results suggest that sequence differences among these rec genes are not responsible for the different rates at which the Nichols and Chicago strains accumulate tprK diversity. To date, no analyses of the expression or function of the rec genes have been performed. Furthermore, the genome sequencing project revealed that T. pallidum Nichols does not carry the genes that encode the RecBCD complex. We are currently examining whether the recBCD genes are also missing in non-Nichols strains. Genes involved in mismatch repair and antirecombination processes, such as the analogs to mutS and mutL, are also being investigated for sequence differences between Nichols and non-Nichols strains that may alter the function of this system.

After many decades of passage, T. pallidum Nichols remains exquisitely infectious for rabbits. Robustly replicating Nichols organisms have a limited repertoire of TprK sequences, and many new V regions revert to the original founder sequence, suggesting that a high degree of TprK heterogeneity is not necessary for the organisms to flourish in the rabbit testis. Furthermore, stretches of V region sequences that were observed in slow-passaged NicholsSea are seen among sequences from Nichols strains maintained in different laboratories. For example, the V1 amino acid motif SAGN that arises at SP2

![FIG. 7. V2 and V6 sequence changes in NicholsSea and clonal Chicago C after 22 (RP22) and 8 (RP8) rapid passages, respectively. Shading is as defined for Fig. 1.](http://iai.asm.org/ on August 15, 2017 by guest)
(Fig. 3) is seen among the NicholsHou sequences (Fig. 1). The KAG motif in V7 is observed in both the Nichols genome sequence (Fig. 1) and NicholsSea SP2 (Fig. 3). V6 (see Fig. S1 in the supplemental material) also displays similarity between sequences that arise with slow passage and sequences from other Nichols strains. This conservation of V region sequences supports the hypothesis that Nichols strains have adapted well to growth in the rabbit testis and express a limited repertoire of optimal TprK sequences.

The vlsE gene in Borrelia burgdorferi, the Lyme disease spirochete, has been shown to undergo sequence change in variable regions using a gene conversion mechanism similar to that postulated for tprK in T. pallidum (27, 28), and the resulting VlsE variants are antigenically distinct from the parent (13). The B. burgdorferi clone that was originally isolated in ticks and maintained by in vitro passage was shown to have a wide variety of variable vlsE sequences (27), and the theoretical limit of diverse vlsE sequences is very high. In contrast, the examination of vlsE sequence changes in B. burgdorferi harvested from long-term-infected mice showed that, at some positions, a significant number of variants contained more “stable” sequences or reverted to the vlsE sequence of the founder clone (23). Additionally, isolates from different geographic regions have an average number of different VlsE sequences similar to that observed in mice infected with clonal populations for only 3 months (23). These divergent observations suggest that there is an in vivo selective advantage for B. burgdorferi expressing only a few VlsE variants, paralleling the data obtained for the TprK V regions in T. pallidum NicholsSea. In contrast, the clonal Chicago C strain, which readily undergoes changes in TprK during intrauterine infection, appears not to exhibit the same in vivo selection phenomenon.

In Giardia lamblia, a reset mechanism has been proposed to be involved in transmission of this pathogen from one murine host to another (25). The antigenic reset of VSP H7 appears not to be due to the expansion of an underrepresented VSP H7 population within an antigenically heterogeneous inoculum but may represent growth selection upon establishment of intestinal infection. Similarly, the restriction of diversity of TprK in Nichols treponemes propagated in testes may reflect the high degree of adaptation of T. pallidum Nichols to this specific environment. However, diverse V region sequences do appear upon slow passage, suggesting that there is allowance for some tprK variability in Nichols. This increase in Nichols tprK sequence diversity may be sufficient to allow the organisms to evade the initial host immune response to reside in other sites (e.g., lymph nodes) during chronic infection. However, as in the B. burgdorferi vlsE gene, the apparent constraints in sequence diversity could suggest a required role for TprK in growth of T. pallidum, with variation occurring only when necessary for immune evasion. This hypothesis is supported by our observation that reversion to the founder sequence was permitted upon passage to immunologically naive rabbits. Ongoing studies in the laboratory are addressing the accumulation of V region diversity and organism tissue localization during long-term infection and the development of antibody responses to the newly generated sequences.

We have demonstrated that the NicholsSea strain accumulates significant tprK sequence diversity only under slow passage, a setting that exposes organisms to the host’s adaptive immune response. During T. pallidum infection, the cellular immune response is directed against the conserved regions of TprK, while the antibody response is targeted to the V regions (15). Furthermore, these antibodies are specific to the particular V region sequences that are expressed by infecting organisms, and immunization with recombinant TprK causes a delay in the growth of T. pallidum with a homologous TprK sequence compared to T. pallidum with a heterologous TprK sequence (14). We speculate that the development of specific antibodies to existing TprK V regions selects for organisms that carry newly arisen V regions and are currently conducting experiments to test this hypothesis. This antigenic variation of tprK may allow the organisms to evade the adaptive immune response to cause chronic infection in the host.

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