

## Molecular Characterization of Common Treponemal Antigens

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A molecular characterization of cross-reactive antigens of *Treponema pallidum* Nichols and *Treponema phagedenis* biotype Reiter that are reactive with normal and syphilitic human sera is described. At least 8 common polypeptides, 14 *T. pallidum*-specific antigens, and 2 *T. phagedenis* biotype Reiter-specific antigens were identified.

Antigenic characterization of *Treponema pallidum* has been hampered by the inability to obtain large quantities of pure, motile, and virulent organisms (1, 9, 10, 21, 22, 28-30). Nonpathogenic treponemes, however, can be grown *in vitro* in pure culture. Thus, in an attempt to obtain information about treponemal antigenic interrelationships, investigators have concentrated their efforts toward the examination of cross-reactive antigens obtained from the nonpathogens. Most of the studies have identified antigens common to both *T. pallidum* Nichols and the host-indigenous, nonpathogenic *Treponema phagedenis* biotype Reiter (referred to here as Reiter). Complement fixation (3-5, 7, 8, 12, 18, 20), gel diffusion (12, 18, 20), fluorescent-antibody (6, 13, 17, 27), crossed immunoelectrophoresis (20, 23-26) and Western blot (15) techniques have revealed the presence of shared protein, polysaccharide, and/or protein-lipopolysaccharide complex antigens. Furthermore, absorption studies have shown that the presence of treponemidal activity in normal human serum against *T. pallidum* and Reiter is stimulated by common *T. pallidum* and Reiter immunogens (P. A. Hanff and J. N. Miller, submitted for publication). However, with the exception of axial filament proteins (12, 21), the common treponemal antigens have not been well characterized in terms of their cellular location or molecular weight. As a prelude to isolation and purification of these particular cell components, we have characterized the common polypeptide antigens of *T. pallidum* and Reiter with normal and syphilitic human sera by using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis followed by electrophoretic transfer of the

polypeptides to nitrocellulose membranes (Western blot technique).

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*T. pallidum* Nichols was maintained and harvested as previously described (16). To increase bacterial yield, animals were given cortisone acetate from days 3 to 10 postinfection (33). Removal of host tissue from treponemal suspensions was accomplished by two differential centrifugations at  $450 \times g$  for 7 min and sedimentation through Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) at  $30,000 \times g$  for 30 min (P. A. Hanff, S. J. Norris, M. A. Lovett, and J. N. Miller, manuscript in preparation). Organisms were suspended in phosphate-buffered saline, divided into portions, and stored at  $-76^\circ\text{C}$ . Reiter treponemes were maintained by transfer in thioglycolate medium without Indicator-135 C (BBL Microbiology Systems, Cockeysville, Md.) and prepared in Spirolate broth (BBL) by the technique described by Stout et al. (30). Organisms were harvested at days 6 to 7, stored overnight at  $4^\circ\text{C}$ , and washed in fluorescent treponemal antibody-absorption buffer (Beckman Instruments, Inc., Fullerton, Calif.) three times over a 3-day period; the final pellets were suspended in one-third volume of buffer and stored at  $-20^\circ\text{C}$ . Organisms and marker proteins (Pharmacia Fine Chemicals) were boiled at  $100^\circ\text{C}$  for 5 min in buffer containing 0.0625 M Tris (pH 6.8), 2% SDS, and 5% 2-mercaptoethanol. *T. pallidum* and Reiter polypeptides were separated on 8 to 20% polyacrylamide slab gels by the discontinuous buffer system of Laemmli (14). Proteins in the gel were electrophoretically transferred to 0.45- $\mu\text{m}$  nitrocellulose paper (Sartorius Filters, Inc., Hayward, Calif.) at 195 mA

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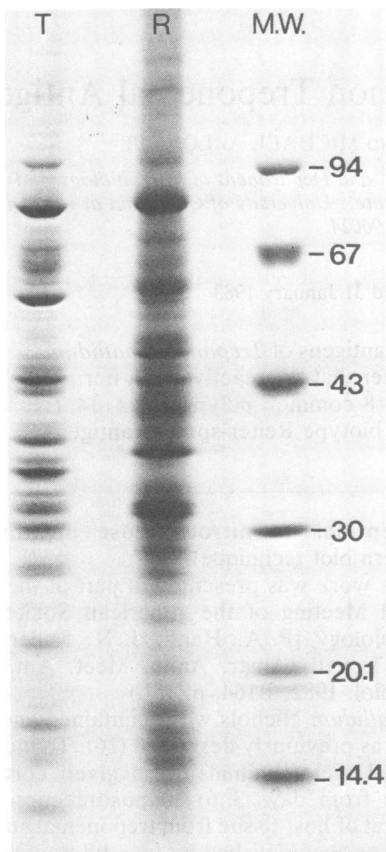


FIG. 1. The polypeptides of *T. pallidum* Nichols (T) and Reiter (R). The proteins of Reiter and Percoll-centrifuged *T. pallidum* were separated by gradient SDS-polyacrylamide gel electrophoresis, and the gel was stained with Coomassie blue. Molecular weight (M.W.) standards ( $\times 10^3$ ) are shown.

for 3 h as previously described (11) in buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol (31). Blots were stained with amido schwarz (31) and cut into 8- to 10-mm strips. The remaining protein-binding sites were blocked with 5% ovalbumin in TSA buffer (50 mM Tris [pH 7.5], 0.9% NaCl, and 0.2% sodium azide). One strip each of *T. pallidum* and Reiter was probed with normal or syphilitic human serum diluted 1:10 or 1:100 in TSA-ovalbumin. Strips were washed with TSA and incubated for 2 h with lactoperoxidase-labeled [ $^{125}$ I]protein A. Unbound label was removed by washing with TSA. Blots were rinsed in water, air-dried, and autoradiographed on MRF-32 clear base X-ray film (Du Pont Corp., Atlanta, Ga.).

The Coomassie blue-stained gel profile of *T. pallidum* and Reiter shown in Fig. 1 identified polypeptides ranging in molecular weight from approximately 150,000 to 14,000. The polypeptide pattern of each organism is distinctive.

The Reiter antigens detected with five normal human sera (NHS) and sera from five patients with dark-field positive primary syphilis are shown in Fig. 2. The normal sera reacted with four polypeptides of 45,000, 40,000, 33,000 and 30,000 daltons. Primary sera detected two additional antigens of 49,000 and 35,500 molecular weight. Although at least five other polypeptides were demonstrable with syphilitic and normal sera, these were not detected by all sera tested.

Figure 3 shows the reaction of serum from patients with secondary syphilis (A,B,C) and early latent syphilis (D,E,F) with blots of *T. pallidum* and Reiter. The three sera from each diagnostic category detected at least 22 *T. pallidum* polypeptides ranging in molecular weight from 94,000 to 15,500 and the six Reiter proteins previously identified with normal and primary syphilitic sera. Two additional common antigens of 82,000 and 73,000 and two unique Reiter antigens of 60,000 and 54,000 molecular weight were also detected.

This report confirms and extends our previous results (11) and those of other investigators (3-8, 12, 13, 15, 17-21, 23-26) who have demonstrated specific and/or cross-reactive treponemal antigens on virulent and nonpathogenic treponemes. Utilizing human syphilitic sera, we have characterized at least eight polypeptide antigens of 82,000, 73,000, 47,000, 45,000, 40,000, 35,500, 33,000, and 30,000 daltons that are common to both *T. pallidum* and Reiter. Similarly, Petersen et al. (26) have identified six cross-reactive antigens utilizing a crossed immunoelectrophoresis technique, and Lukehart et al. (15), using pooled rabbit anti-*T. pallidum* antiserum, have recently defined five common antigens of 69,000, 48,000, 40,000, 38,000, and 34,000 daltons.

Furthermore, we have identified 14 *T. pallidum*-specific and 2 Reiter-specific polypeptides. In contrast, Pedersen et al. (23, 24) and Lukehart et al. (15) detected only three *T. pallidum*-specific antigens. Although the reasons for these differences are unclear, our ability to detect as many as 22 treponemal antigens with human and experimental syphilitic sera (11) may be related to the fact that the antigen concentrations used in our studies were approximately 20- to 100-fold greater than that utilized in Lukehart's study (S. Lukehart, personal communication). It is also likely that our Western blot technique is a more sensitive antigen detection system than crossed immunoelectrophoresis. Whereas we have shown that the 45,000-, 40,000-, 33,000-, and 30,000-molecular-weight antigens of Reiter and *T. pallidum* are detected by immunoglobulin G present in NHS (11), reactivity with NHS was not demonstrable by Pedersen et al. (23, 24).

Although the cellular location of the common

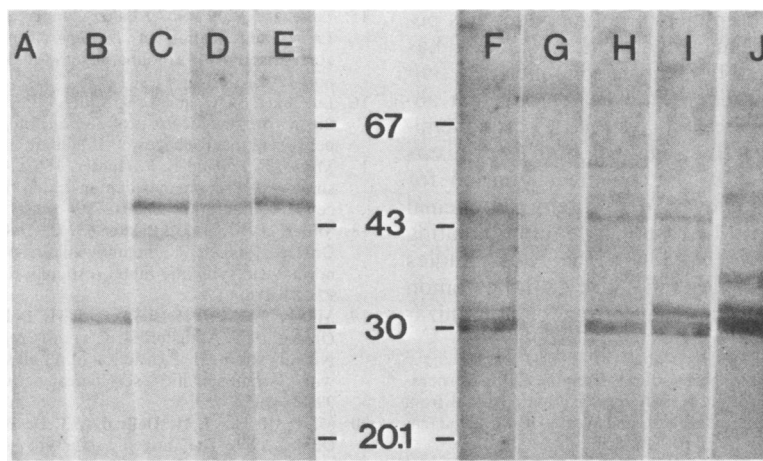


FIG. 2. The reaction of normal human (A to E) and primary syphilitic (F to J) sera with polypeptides of Reiter. A blot of *T. phagedenis* polypeptides was prepared with an 8 to 20% gradient SDS-polyacrylamide gel as described in the text. The blot was cut into 10-mm-wide strips, which were incubated for 16 h with sera diluted 1:10 in TSA buffer containing 5% ovalbumin. Antigenic proteins were detected with [ $^{125}$ I]protein A and autoradiographed with MRF-32 X-ray film (Du Pont Co.). The numbers refer to the molecular weight ( $\times 10^3$ ) of protein standards that were included but are not shown.

treponemal antigens has yet to be determined, there is evidence suggesting that axial filaments are among the shared antigens (12, 21). Additionally, the 30,000- and 33,000-dalton polypeptides described in this study are within the

reported molecular weight range for Reiter axial filaments (2). Studies are in progress to define the exact cellular location and the identity of the common and specific polypeptide antigens.

The finding of NHS reactivity with four of the

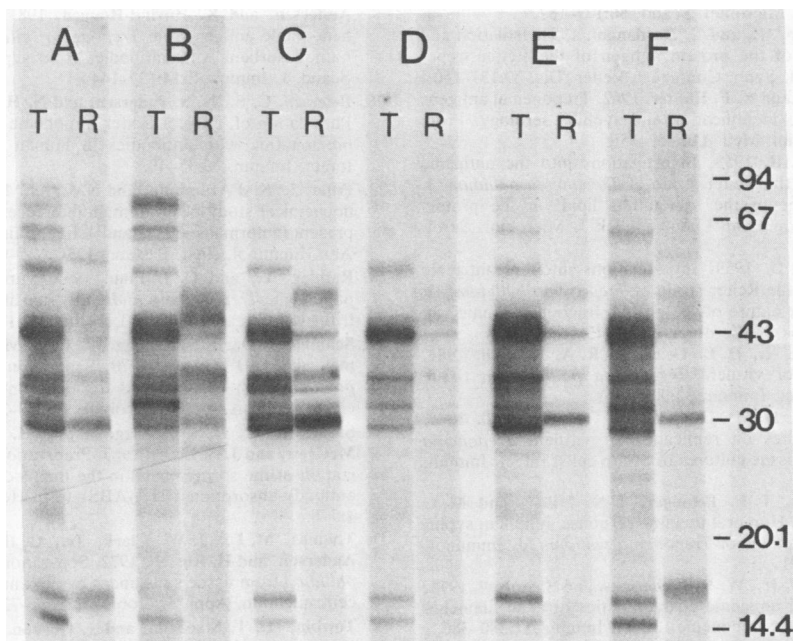


FIG. 3. The reaction of sera from patients with secondary (A to C) and early latent (D to F) syphilis with blots of *T. pallidum* (T) and Reiter (R). The blots were prepared by 8 to 20% gradient SDS-polyacrylamide gel electrophoresis and electrophoretic transfer, and blot strips were incubated for 16 h with sera diluted 1:100. The numbers represent the molecular weight ( $\times 10^3$ ) of protein standards that were included but are not shown.

eight common treponemal polypeptides has potential vaccinogenic significance, since it has been shown that NHS has treponemicidal activity against *T. pallidum* and Reiter and that absorption with Reiter may remove the treponemicidal factors active against both organisms (P. A. Hanff and J. N. Miller, submitted for publication). However, nonprotein treponemal antigens also may be responsible for stimulating treponemicidal activity in NHS. Thus, studies designed to isolate and purify the common treponemal antigens from Reiter for immunization have been planned.

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