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NOTES

Pathogen Specificity of *Treponema pallidum* subsp. *pallidum* Integral Membrane Proteins Identified by Phase Partitioning with Triton X-114

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The antigenically conserved proteins of *Treponema pallidum* subsp. *pallidum* and four nonpathogenic cultivatable treponemes were investigated by phase partitioning with the nonionic detergent Triton X-114 and immunoblot analysis. None of the *T. pallidum* integral membrane proteins identified by phase partitioning (detergent-phase proteins) appeared to be antigenically related to proteins of the nonpathogens. Protease-resistant material similar to lipopolysaccharide was identified in the detergent phase from *T. phagedenis* biotype Reiter but was not detected in *T. pallidum*.

Molecular analysis of *Treponema pallidum* subsp. *pallidum* (*T. pallidum*), the etiologic agent of venereal syphilis, has been impeded by the inability to cultivate the pathogen on artificial medium. One strategy for circumventing this problem has been to purify antigenically conserved molecules from cultivatable nonpathogenic treponemes, particularly *T. phagedenis* biotype Reiter (*T. phagedenis*), to characterize the analogous constituents of *T. pallidum* (15, 16). In view of the extremely limited DNA homology that exists between the pathogenic and the nonpathogenic treponemes (14, 18), the general applicability of this strategy to the investigation of *T. pallidum* proteins important in syphilis pathogenesis remains unclear. Recently, we (17) demonstrated that phase partitioning with the nonionic detergent Triton X-114 (2) could be used to isolate from *T. pallidum* a group of highly immunogenic integral membrane proteins (designated the detergent-phase proteins). In the present study, we used this technique to investigate the antigenic relationships between these important immunogens and the proteins from four species of cultivatable nonpathogenic treponemes.

T. pallidum Nichols strain was propagated in male New Zealand White rabbits and was purified by Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) density gradient centrifugation (6). *T. phagedenis* biotype Reiter, *T. scoliodontum*, *T. vincenti*, and *T. denticola* were grown as described previously (1, 18) and were washed in phosphate-buffered saline, pH 7.4. Portions containing either 5×10^9 *T. pallidum* or an equal number of *T. phagedenis* were extracted with 2% Triton X-114 (Sigma Chemical Co., St. Louis, Mo.) in phosphate-buffered saline for 20 min at 4°C. Insoluble material was removed by centrifugation, and the separated detergent and aqueous phases were washed repeatedly (17). Detergent-phase proteins were acetone precipitated and resuspended in 50 μl of phosphate-buffered saline. The aqueous phases were dialyzed against distilled water in Spectra/Por 6 (Spectrum Medical Industries, Los Angeles, Calif.) 2,000-molecular-weight-cutoff dialysis tubing and concentrated on a Speed-Vac apparatus (Savant Instruments, Inc., Farmingdale, N.Y.).

Except when noted, samples for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were boiled for 10 min in final sample buffer containing 2-mercaptoethanol and electrophoresed on 4% stacking and 12.5% separating gels, both with 2.6% bisacrylamide cross-linking (10). Gels were stained or were electrotransferred to nitrocellulose sheets (0.2-μm pore size; Schleicher & Schuell, Inc., Keene, N.H.). Immunoblots were incubated in 1:100 dilutions of pooled human syphilitic sera or rabbit antisera directed against one of the nonpathogenic treponemes, probed with ^{125}I-labeled staphylococcal protein A, and autoradiographed at −70°C. Treponemal material analogous to lipopolysaccharide (LPS) was identified by digesting 10^9 whole organisms or 10-μl portions of their detergent phases with 25 μg of proteinase K (Sigma) as described previously (4). After proteinase K digestion, samples were loaded directly into the stacking gels for SDS-PAGE and silver staining (4, 19). As a control, 500 μg of purified *Escherichia coli* J5 LPS (List Biological Laboratories, Campbell, Calif.) was phase separated in 1 ml of 2% Triton X-114; 1 μl of the detergent phase was used for SDS-PAGE.

The detergent phase from *T. pallidum* contained a number of polypeptide antigens, including the well-characterized 47-kilodalton (kDa) major immunogen of the organism (Fig. 1A and B) (17). The polypeptide and antigenic profiles of the *T. phagedenis* detergent phase were distinctly different from those of *T. pallidum*; the absence of an abundant 47-kDa protein was particularly noteworthy (Fig. 1A). The *T. phagedenis* detergent phase also contained highly antigenic proteinase K-resistant material that formed a stepladder pattern similar to that of smooth LPS (Fig. 1B). The presence of such material in *T. phagedenis* has been noted previously (4, 8).

Cross-reactive proteins from *T. phagedenis* and *T. pallidum* were characterized by immunoblotting whole-cell lysates, Triton X-114-insoluble material, and the detergent and

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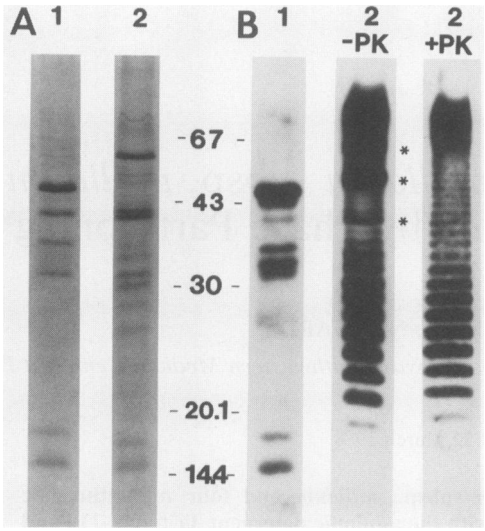

FIG. 1. Comparison of detergent phases from *T. pallidum* and *T. phagedenis* biotype Reiter. (A) SDS-12.5% polyacrylamide gel stained with Coomassie brilliant blue. Lanes: 1, *T. pallidum* detergent phase; 2, *T. phagedenis* detergent phase. (B) Immunoblot analysis. Lanes: 1, *T. pallidum* detergent phase reacted with pooled human syphilitic sera; 2, *T. phagedenis* detergent phase reacted with rabbit anti-*T. phagedenis* serum before (−PK) and after (+PK) treatment with proteinase K (asterisks indicate discernible proteinase K-sensitive *T. phagedenis* detergent-phase proteins). Molecular size standards (in kilodaltons) are shown between the panels.

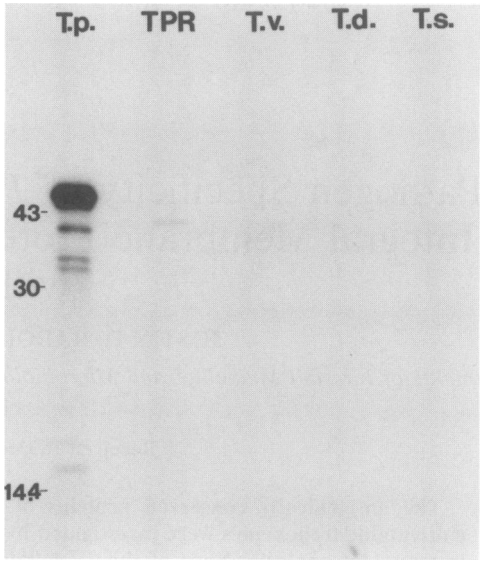

FIG. 3. Immunoblot analysis of rabbit antiserum generated against the entire *T. pallidum* detergent phase reacted with whole-cell lysates of *T. pallidum* (T.p.), *T. phagedenis* biotype Reiter (TPR), *T. vincenti* (T.v.), *T. denticola* (T.d.), and *T. scoliodontum* (T.s.). Molecular size standards (in kilodaltons) are shown on the left.

aqueous phases of *T. phagedenis* against pooled human syphilitic sera and by immunoblotting the corresponding fractions of *T. pallidum* against rabbit anti-*T. phagedenis* antiserum. The majority of antigenically conserved proteins in both organisms were contained in the Triton X-114-insoluble material (Fig. 2A and B). A cross-reactive protein of approximately 47 kDa was identified in both organisms. For *T. pallidum*, this protein partitioned into the aqueous phase (Fig. 2B). Neither the *T. pallidum* nor the *T. phagedenis* detergent-phase antigens reacted with antisera directed against the other organism. Rabbit antisera generated against two other nonpathogens, *T. scoliodontum* and *T. denticola*, also failed to react with the *T. pallidum* detergent-phase proteins, but they did recognize several *T. phagedenis* detergent-phase proteins and the LPS-like material (data not shown). As a final test of pathogen specificity, rabbit antisera generated against the entire *T. pallidum* detergent phase were immunoblotted against a panel of nonpathogenic treponemes. With the exception of a 39-kDa *T. phagedenis* antigen not recognized by the preimmune serum, significant reactivity against the nonpathogens was not detected (Fig. 3).

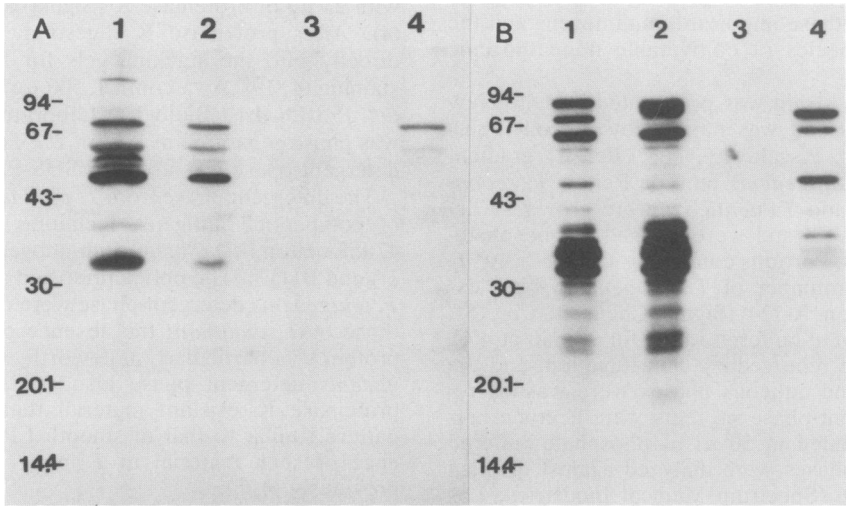

FIG. 2. Immunoblot analysis of *T. phagedenis* (A) and *T. pallidum* (B) fractions reacted with pooled human syphilitic sera (A) and rabbit antiserum directed against *T. phagedenis* (B). Lanes: 1, whole-cell lysates; 2, Triton X-114-insoluble material; 3, detergent phase; 4, aqueous phase. Molecular size standards (in kilodaltons) are shown on the left of each panel.

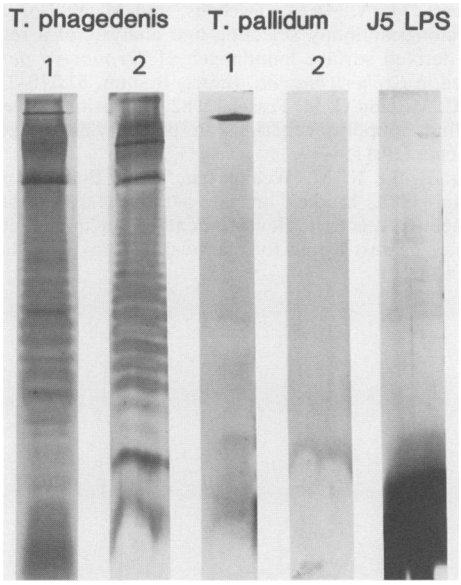

FIG. 4. Proteinase K-resistant material in *T. pallidum* and *T. phagedenis*. Whole-cell lysates of *T. pallidum* and *T. phagedenis* (lanes 1) and their respective detergent phases (lanes 2) were digested with proteinase K and analyzed by SDS-PAGE and silver staining. A 1-μl sample from the detergent phase of phase-partitioned *E. coli* J5 LPS (right lane) is included.

Conflicting evidence has been obtained by other investigators with regard to the presence of LPS in the pathogenic treponemes (4, 5, 7, 20). The ability of phase partitioning to identify material similar to LPS in *T. phagedenis* suggested that it could be used to determine more definitively whether an analogous substance exists in *T. pallidum*. Silver-stained proteinase K digests of whole *T. phagedenis* and *T. phagedenis* detergent phase contained material which appeared to be the same as that identified by immunoblotting (Fig. 1B and 4). Proteinase K-digested whole *T. pallidum* contained a high-molecular-weight component, the protease-resistant 4D antigen (4), and diffusely staining material that migrated near the gel dye front (Fig. 4). However, the *T. pallidum* detergent phase was entirely devoid of protease-resistant material. Purified *E. coli* J5 LPS partitioned into the detergent phase under the same conditions (Fig. 4).

The above experiments provided strong evidence to support the pathogen specificity of the entire group of *T. pallidum* integral membrane proteins isolated by Triton X-114 phase partitioning (17). The putative pathogen specificity of the 47-kDa major immunogen, in particular, has been a subject of considerable debate in the literature (1, 3, 9, 11, 12, 13). Phase partitioning has enabled resolution of this issue by demonstrating that *T. pallidum* contains two 47-kDa protein antigens with substantially different solubility properties. The cross-reactive protein is hydrophilic and probably corresponds to the *T. phagedenis* protein designated TR-c by Petersen and co-workers (15).

Because these findings are limited to those membrane proteins of *T. pallidum* that can be extracted with Triton X-114, they leave open the possibility that antigenically related, but as yet unidentified, integral membrane proteins do exist between the pathogenic and nonpathogenic treponemes. Nevertheless, the absence of antigenic relatedness for this important subset of *T. pallidum* membrane immunogens indicates that, as opposed to the suggestion of other investigators (15), cross-reactive molecules purified from the nonpathogenic treponemes are unlikely to have vaccinogenic potential. The common treponemal proteins most likely represent a small number of conserved molecules that subserve basic structural or metabolic functions or both in these organisms.

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