A New Attempt To Distinguish Serologically the Subspecies of *Treponema pallidum* Causing Syphilis and Yaws

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In an effort to serologically differentiate syphilis from yaws, 69 monoclonal antibody species raised against *Treponema pallidum* subsp. *pallidum* were tested by immunoblotting for their reactivity with *Treponema pallidum* subsp. *pertenue*. All monoclonal antibodies reacted with antigens with the same molecular weight of both subspecies. Furthermore, no differences in reactivity between sera from yaws patients and from syphilis patients were found by Western blot (immunoblot) analysis of cell lysates of *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*. We tried to exploit the only known molecular difference between the subspecies. The subunits of the 190-kilodalton multimeric proteins TpF1 and TyF1 of *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*, respectively, have previously been shown to differ in one amino acid residue at position 40. In this study, no difference was found in immunoreactivity of TpF1 or TyF1 with either syphilis sera or yaws sera. Synthetic peptides based on the sequence of TpF1 and of TyF1 were used in an enzyme-linked immunosorbent assay with syphilis sera and yaws sera. Again, no difference in reactivity between the *T. pallidum* subsp. *pallidum*- and *T. pallidum* subsp. *pertenue*-derived peptides was observed.

Infection of humans and laboratory animals with *Treponema pallidum* subsp. *pallidum* results in a strong humoral response. By means of sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting, up to 22 immunogenic polypeptides of *T. pallidum* subsp. *pallidum* have been detected (5). The antibody response of different syphilitic individuals and animals is remarkably uniform with regard to the specificity of antibodies reacting with the various polypeptides, as determined by immunoblotting or radioimmunoprecipitating techniques (1, 7, 8, 15, 16, 19, 23, 25, 28). A number of the *T. pallidum* subsp. *pallidum* antigens have been further characterized with monoclonal antibodies (MAbs) and expression of the antigens in *Escherichia coli* (2-4, 9, 17, 24, 29, 30, 32, 34, 36, 37, 39, 43, 44).

Treponema pallidum subsp. *pertenue* is the causative agent of yaws, and, like *T. pallidum* subsp. *pallidum*, this bacterium cannot be cultivated in vitro. The two subspecies differ markedly in their mode of transmission and in the type of disease they cause. They also differ in their pathogenicity for experimental animals (18, 41). Nevertheless, no clear difference in morphology or antigenic makeup between the subspecies has been detected to date (6, 7, 32, 38, 40). Attempts to find differences between antigens of both subspecies have been unsuccessful, even by means of immunoblot analysis of polypeptides separated by two-dimensional electrophoresis. Only minor quantitative differences in polypeptide profiles of the two subspecies have been observed. Sera from yaws patients are reactive in the currently used cardiolipin- and *T. pallidum* subsp. *pallidum*-based serological tests for syphilis (12, 22, 27). Therefore, no serological differentiation between syphilis and yaws can be made on the basis of routine laboratory tests. This is unfortunate, because follow-up of treatment of these diseases differs greatly because of differences in pathogenesis and transmis-

sion. For example, in contrast to syphilis, neither in utero transmission nor neurovascular disorders are encountered in yaws. Especially in areas where both treponematoses are endemic, it is important to have a laboratory tool to differentiate these diseases.

In this report, we describe a further attempt to identify antigenic differences between *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*. We have analyzed, by means of immunoblotting, the reactivity of a large number of human syphilis sera and yaws sera with polypeptides of *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*. Furthermore, the reactivity with *T. pallidum* subsp. *pertenue* of a large number of MAbs raised against *T. pallidum* subsp. *pallidum* is reported.

Recently, we described a marked difference in electrophoretic mobility between a proteinase K-treated antigen derived from *T. pallidum* subsp. *pallidum*, TpF1, and a homologous antigen from *T. pallidum* subsp. *pertenue*, TyF1 (30). The amino acid sequences of the 19-kilodalton (kDa) subunit polypeptides of TpF1 and TyF1 were established, and they differed in a single residue at position 40 in the molecule. TpF1 has a glutamine and TyF1 has an arginine at this position. So far, this is the only molecular difference known between strains of these subspecies.

In this study, we tried to exploit the one amino acid difference between TpF1 and TyF1 by searching for an antigenic difference between these proteins. Such an antigenic difference could provide the basis of a serological method for the differentiation of syphilis and yaws.

MATERIALS AND METHODS

Antigens. *T. pallidum* subsp. *pallidum* Nichols and *T. pallidum* subsp. *pertenue* CDC 2575 were maintained by serial passage in rabbit testes (30). Cells of *T. pallidum* subsp. *pertenue* Gauthier were kindly provided by P. Hindersson, Statens Serum Institute, Copenhagen, Denmark.

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Treponemes were purified from testicular tissue by urografin gradient centrifugation (43). Recombinant *E. coli* K-12 strains carrying plasmids pRIT7170 and pREN1032 were used for production of the *T. pallidum* subsp. *pallidum* protein TpF1 and *T. pallidum* subsp. *pertenue* protein TyF1, respectively (30). Cell suspensions in 50 mM Tris-5 mM EDTA (pH 8) with an optical density at 600 nm of 20 were sonicated on ice with short bursts at 80 to 100 W. To obtain the 95- and 115-kDa proteinase K-resistant residues of TpF1 and TyF1 (95-kDa TpF1 and 115-kDa TyF1, respectively), the sonicated extracts were incubated with proteinase K (100 µg/ml) in 50 mM Tris hydrochloride (pH 8.3) containing 2% sodium dodecyl sulfate for 4 h at 37°C. The digestion was terminated by adding phenylmethylsulfonyl fluoride to a final concentration of 100 µM. Two synthetic peptides, PepA and PepB, containing 15 amino acids were obtained from E. Freund, Hubrecht Laboratory, Utrecht, The Netherlands. PepA has the amino acid sequence 33 to 47 of TpF1, AICEQLRQHVADLGV, and PepB has the homologous sequence of TyF1, AICEQLRRHVADLGV (30). One milligram of either synthetic peptide was conjugated to 7.5 mg of bovine serum albumin (fraction V; Sigma) with glutaraldehyde.

MAbs. MAbs against *T. pallidum* subsp. *pallidum* used in this study are listed in Table 1 and were kindly provided by different research groups as shown. The values of the molecular weights of the treponemal antigens were used as suggested by Norris et al. (33), except for TmpA, where we used the value of 42 kDa (37). MAbs C18 to C810 directed against the 35-kDa protein TmpC (43) and MAbs B52 to B712 directed against the 34-kDa protein TmpB (43) were obtained from mice immunized repeatedly with purified recombinant TmpC (L. M. Schouls, unpublished data) and recombinant TmpB (37), respectively, and were kindly provided by A. Osterhaus (Bilthoven, The Netherlands).

Human sera. Syphilis sera were obtained from patients at the Clinic for Sexual Transmitted Diseases of the University Hospital Dijkzigt, Rotterdam, The Netherlands. Patients were classified into groups of primary syphilis, secondary syphilis, late latent syphilis, or treated syphilis as described by IJsselmuiden et al. (21). Sera from yaws patients were obtained from several countries where yaws is endemic. The yaws patients were selected on the basis of clinical examination by having typical yaws lesions and by positive serology in the classical syphilis tests. A total of 15 serum samples was obtained from S. Sadal from 1984 to 1985 from children in Surinam with clinical symptoms of early yaws. A total of 24 serum samples originated from children in Ghana and were obtained by T. van der Werf in 1987. Of these serum samples, 15 were from children with early yaws and 9 were from healthy children with no known history of yaws; the latter sera were taken as negative controls from a yaws-endemic area. Ten yaws serum samples were obtained from S. Larsen, Centers for Disease Control, Atlanta, Ga. A total of 103 serum samples were obtained in 1988 from yaws patients in West Sumatra, Indonesia (G. T. Noordhoek, H. J. H. Engelkens, J. Judanarso, J. van der Stek, G. N. M. Aelbers, J. J. van der Sluis, J. D. A. van Embden, and E. Stolz, submitted for publication). After collection, the serum samples were stored at -20°C. All serum samples were tested in the classical syphilis tests. The *T. pallidum* hemagglutination assay was performed with a serum dilution of 1/80; hemagglutination was judged from "-" to 2+ (TPHA test Medac, Federal Republic of Germany). The Venereal Disease Research Laboratory test was performed with two-fold serum dilutions (42). The fluorescent treponemal anti-

body absorption test was performed semiquantitatively with a serum dilution of 1/5 and was scored from "-" to 4+ (42).

Rabbit antisera. Rabbit antisera against *T. pallidum* subsp. *pallidum*, *T. pallidum* subsp. *pertenue*, TpF1, and TyF1 were obtained as described previously (30). Rabbit antisera against synthetic peptides PepA and PepB were obtained after two subcutaneous injections with 100 µg of the bovine serum albumin-conjugated peptides. The first injection was with Freund complete adjuvant, and then 3 weeks later, a booster injection was given with Freund incomplete adjuvant. The rabbits were bled 10 days after the last injection.

Immunological assays. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting (immunoblotting) were done as described previously (30, 43). Thirteen percent polyacrylamide or a gradient from 6 to 18% polyacrylamide was used for the gels. The enzyme-linked immunosorbent assay (ELISA) was carried out as described by IJsselmuiden et al. (22) with 1 µg of conjugated peptide per well. Coating of ELISA wells with conjugated peptide was performed in sodium bicarbonate buffer (pH 9.5) for 16 h at 37°C.

Two sets of overlapping nonapeptides were synthesized on the basis of amino acid sequences of TpF1 and TyF1, respectively. These sets covered the amino acid sequences between residues 32 and 48 of TpF1 and TyF1 (see Fig. 4A) by using the Pepsan method of solid-phase synthesis on activated polyethylene rods arrayed in a microdilution plate pattern (13, 14). After synthesis and removal of the amino acid side-chain protecting groups, the peptides remained attached to the rods for subsequent analysis of their reactivity with antibodies by an ELISA.

RESULTS

Reactivity of anti-*T. pallidum* subsp. *pallidum* MAbs with *T. pallidum* subsp. *pertenue*. MAbs, raised against at least 15 different *T. pallidum* subsp. *pallidum* Nichols protein antigens, were tested for their ability to bind to *T. pallidum* subsp. *pertenue* Gauthier antigens. This was done by Western blotting of solubilized whole cells of *T. pallidum* subsp. *pertenue*. Immunoblots of *T. pallidum* subsp. *pallidum* served as a control.

All of the *T. pallidum* subsp. *pallidum*-reactive MAbs listed in Table 1 were also reactive with *T. pallidum* subsp. *pertenue*, and the molecular weights of the *T. pallidum* subsp. *pertenue* Gauthier proteins recognized were indistinguishable from those of *T. pallidum* subsp. *pallidum* (Fig. 1). Only one slight difference in banding pattern of *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* was observed; the MAbs 2B11, 2D7, and 1-14M1, raised against the TmpA antigen, all reacted on a blot of *T. pallidum* subsp. *pertenue* Gauthier with a protein doublet corresponding to 42 and 40 kDa (Fig. 1, lane 5). In contrast, *T. pallidum* subsp. *pallidum* Nichols showed only a slight band of the TmpA antigen at 42 kDa, as was established previously (17). The *T. pallidum* subsp. *pertenue* doublet was not observed on blots loaded with antigen of *T. pallidum* subsp. *pertenue* CDC 2575 (data not shown).

Reactivity of sera from yaws and syphilis patients with separated *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* proteins. Previous studies on the reactivity of antibodies to polypeptides of *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* have been done with sera from rabbits infected with *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*, respectively (6). To investigate whether human sera from yaws and syphilis patients would

TABLE 1. MAbs directed against *T. pallidum* subsp. *pallidum* antigens

MAb	Mol size of antigen recognized (kDa)	Antigen designation	Origin or reference no.
C-12	80, 47		S. Lukehart, Seattle, Wash.
HATR1.27	70		P. Hinderesson, Copenhagen
HATR1.16	60, 24 to 28	Tp4, TpE	P. Hinderesson, Copenhagen
HATR1.20	60	Tp4	P. Hinderesson, Copenhagen
HATR1.24	60	Tp4	33; P. Hinderesson, Copenhagen
HATR1.7	47	TpS	P. Hinderesson, Copenhagen
HATR1.5	47	TpS	P. Hinderesson, Copenhagen
HATR1.4	47	TpS	33; P. Hinderesson, Copenhagen
HATR1.1	47	TpS	P. Hinderesson, Copenhagen
AD12	47	TpS	2; M. J. Bailey, Birmingham, England
AD5	47	TpS	2, 33; M. J. Bailey, Birmingham
AH9	47	TpS	2; M. J. Bailey, Birmingham
C3E5	47	TpS	33, 43
4a	47	TpS	29, 33
11E3	47	TpS	23, 33
8G2	47	TpS	32, 33
H9-1	47	TpS	20
2B11	42	TmpA	2, 33
2D7	42	TmpA	2, 33
1-14M1	42	TmpA	33, 43
25	40		M. J. Bailey, Birmingham
33a	40		4, 33
22	40		M. J. Bailey, Birmingham
CC9	37	Axial filament	3
IB8	37	Axial filament	3
HC2	37	Axial filament	3
C18	35	TmpC	L. M. Schouls, unpublished data
C31	35	TmpC	L. M. Schouls, unpublished data
C33	35	TmpC	L. M. Schouls, unpublished data
C37	35	TmpC	L. M. Schouls, unpublished data
C58	35	TmpC	L. M. Schouls, unpublished data
C118	35	TmpC	L. M. Schouls, unpublished data
C218	35	TmpC	L. M. Schouls, unpublished data
C317	35	TmpC	L. M. Schouls, unpublished data
C321	35	TmpC	33; L. M. Schouls, unpublished data
C322	35	TmpC	L. M. Schouls, unpublished data
C410	35	TmpC	L. M. Schouls, unpublished data
C518	35	TmpC	L. M. Schouls, unpublished data
C523	35	TmpC	L. M. Schouls, unpublished data
C613	35	TmpC	L. M. Schouls, unpublished data
C711	35	TmpC	L. M. Schouls, unpublished data
C718	35	TmpC	L. M. Schouls, unpublished data
C810	35	TmpC	L. M. Schouls, unpublished data
HATR1.2	35		P. Hinderesson, Copenhagen
B1/D4	35		S. Lukehart, Seattle
A11	35		S. Lukehart, Seattle
15	35, 29	TmpC	4
HATR1.29	34	TmpB	P. Hinderesson, Copenhagen
B52	34	TmpB	J. D. A. van Embden
B222	34	TmpB	J. D. A. van Embden
B312	34	TmpB	J. D. A. van Embden
B712	34	TmpB	33; J. D. A. van Embden
2a	34		29, 33
HATR1.14	30		P. Hinderesson, Copenhagen
HATR.19	30		P. Hinderesson, Copenhagen
HATR1.21	30		P. Hinderesson, Copenhagen
SC12	30, 32		2
JD11	30		2
3B5	29 to 35	TpD	33, 39
9B12	29 to 35	TpD	33, 39
HATR1.2	29 to 35	TpD	P. Hinderesson, Copenhagen
10c	29 to 35	TpD	2, 33
17a	29 to 35	TpD	M. J. Bailey, Birmingham
HATR1.26	29 to 35	TpD	P. Hinderesson, Copenhagen
19b	29 to 35	TpD	4, 33
HATR1.25	24 to 28	TpE	33; P. Hinderesson, Copenhagen
HATR1.17	21	TpT	33; P. Hinderesson, Copenhagen
1b	15.5		29
3	14		M. J. Bailey, Birmingham

FIG. 1. Representative immunoblots of *T. pallidum* subsp. *pallidum* Nichols (A) and *T. pallidum* subsp. *pertenue* Gauthier (B) antigens incubated with various MAbs. Lanes: 1, rabbit antiserum against *T. pallidum* subsp. *pallidum*; 2, MAb HATR1-27; 3, HATR1-20; 4, H9-1; 5, 1-14M1; 6, 33a; 7, CC9; 8, 2b; 9, C613; 10, B712; 11, HATR1-14; 12, SC12; 13, HATR1-26; 14, HATR1-25; 15, 1b. Numbers of the left indicate molecular sizes (in kilodaltons) of various *T. pallidum* subsp. *pallidum* antigens.

react differently with any antigen from either *T. pallidum* subsp. *pertenue* or *T. pallidum* subsp. *pallidum*, proteins from the Gauthier and the Nichols strains were subjected to Western blotting. Blots were developed with 42 serum specimens from yaws patients and 18 serum samples from syphilis patients at various stages of the disease. The group of yaws sera was composed of 15 serum samples from Surinam, 3 from Indonesia, 14 from Ghana, and 10 from the Centers for Disease Control.

No significant differences in banding pattern were found between blots loaded with either *T. pallidum* subsp. *pallidum* or *T. pallidum* subsp. *pertenue* antigen. Representative immunoblots are shown in Fig. 2. Furthermore, no particular protein bands could be distinguished which were specifically reactive with either yaws sera or syphilis sera.

A slight strain difference was found in the 24- to 28-kDa antigen TpE. The latter antigen from *T. pallidum* subsp. *pertenue* Gauthier has a smaller apparent molecular mass than does TpE derived from *T. pallidum* subsp. *pallidum* Nichols (Fig. 2C). However, the apparent molecular mass of TpE from *T. pallidum* subsp. *pertenue* CDC 2575 was identical to that of the Nichols strain (data not shown). Therefore, this slight strain-dependent difference in TpE does not seem to be a subspecies-specific property.

A small number of serum samples was also tested on a Western blot loaded with antigen of *T. pallidum* subsp. *pertenue* CDC 2575. Reaction patterns seen on this blot did not differ from those loaded with the other two antigens (data not shown).

Sera with a weak reactivity in the fluorescent treponemal

FIG. 2. Representative immunoblots of lysates of *T. pallidum* subsp. *pallidum* Nichols (A), *T. pallidum* subsp. *pertenue* Gauthier (B), and *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* (lanes a and b, respectively) (C), incubated with human sera. Lanes: 1, control serum from a healthy individual; 2 and 3, primary syphilis patients; 4, 5, 16, and 17, secondary syphilis; 6 and 7, late latent syphilis; 8 and 9, treated syphilis; 10 through 15 and 18 through 20, yaws patients. Lanes a and b in panel C, *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*, respectively. The gels for panels A and B were run on 13% polyacrylamide; for panel C, a gradient polyacrylamide gel was used. Numbers on the left indicate molecular sizes (in kilodaltons) of various *T. pallidum* subsp. *pallidum* antigens. The positions of the double band of the 42-kDa TmpA antigen and the 24- to 28-kDa TpE antigen are indicated with arrows on the right.

antibody absorption test (not more than 1+) usually reacted only with a limited number of polypeptides on the Western blots (Fig. 2, lanes 8, 9, and 13).

Reactivity of sera with proteinase K-treated TpF1 and TyF1. Previously, we showed that proteolysis of TpF1 and TyF1 with proteinase K resulted in fragments with apparent differences in molecular mass. These were 95 kDa for TpF1 and 115 kDa for TyF1 (30). In order to investigate the possible presence of subspecies-specific epitopes on these antigens, Western blots of 95-kDa TpF1 and 115-kDa TyF1 were tested with sera from syphilis patients with different stages of the disease and with sera from yaws patients from different countries.

All sera reactive with 95-kDa TpF1 were also reactive with 115-kDa TyF1, suggesting that the single amino acid difference between the *T. pallidum* subsp. *pallidum*- and the *T. pallidum* subsp. *pertenue*-derived antigens does not result in a difference in major epitopes on these molecules. The reactivity of syphilis sera with these two antigens ranged from about 33% for primary syphilis and treated syphilis to 60 to 70% for secondary and latent syphilis patients. In the yaws sera, reactive antibodies were found in 8% for sera from Ghana to 40% for sera from Indonesia. These data indicate that the various individuals differ greatly in their immune response to this antigen.

Reactivity of sera to TpF1- and TyF1-specific peptides. The TpF1 polypeptide subunit differs from the TyF1 polypeptide subunit in a glutamine residue at position 40 instead of an arginine residue, as in TyF1. To determine whether this difference results in a difference in linear antigenic determinants on these molecules, two peptides of 15 amino acids identical with the region spanning amino acid 33 to 47 of TpF1 and TyF1 were synthesized with either a glutamine or an arginine at position 40. These peptides were designated PepA and PepB, respectively. Both peptides, conjugated to bovine serum albumin, were used in an ELISA to investigate whether syphilis sera or yaws sera would contain antibodies specific for either TpF1 or TyF1. Although the optical density values in the ELISA were rather low, the results were reproducible. No significant difference in reactivity to PepA and PepB was observed with either group of sera (Fig. 3). On average, syphilis sera were more reactive with the synthetic peptides than were yaws sera. Sera from secondary syphilis patients were most reactive, and all of these sera displayed optical density values higher than that of the control sera (Fig. 3).

To further investigate the region of TpF1 and TyF1 spanning amino acid residues 32 to 48 for the presence of a subspecies-specific epitopes, we synthesized two sets of overlapping nonapeptides from residues 32 to 40, 33 to 41, etc., corresponding to the sequences of TpF1 and TyF1 (Fig. 4A). Rabbit antisera raised against *T. pallidum* subsp. *pallidum*, *T. pallidum* subsp. *pertenue*, TpF1, TyF1, five serum samples from yaws patients, and five serum samples from syphilis patients were tested in an ELISA with the two sets of overlapping nonapeptides as antigens. None of the serum samples reacted specifically with any of the nonapeptides of either set (Fig. 4B, panels 1 to 6). Only the sera raised against the synthetic peptides pepA and pepB were reactive with nonapeptides, starting with residues 39 and 40 of both series (Fig. 4B, panels 7 and 8).

DISCUSSION

Among the 69 anti-*T. pallidum* subsp. *pallidum* MAb tested, we failed to find an antibody species that was not

FIG. 3. Reactivity of antibodies from syphilis patients and yaws patients with the synthetic peptides PepA (A) and PepB (B). Each dot represents the optical density value of an individual serum sample obtained in an ELISA. Abbreviations: L1, primary syphilis; L2, secondary syphilis; LL, late latent syphilis; NHS, sera from healthy individuals.

reactive with *T. pallidum* subsp. *pertenue*. In some cases, we observed a slight difference in staining intensity on Western blot between *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* antigens, suggesting either a different level of expression of the antigen or different binding constants. This is similar to the results reported by Lukehart et al. (24) and Marchitto et al. (26), who found that some MABs had a different reactivity with *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* in an immunofluorescence assay. With MABs and patient sera, significant strain differences were observed only in the 42-kDa TmpA and the 24- to 28-kDa TpE antigen. The anti-TmpA MABs reacted with only one protein band in the case of *T. pallidum* subsp. *pallidum* Nichols and *T. pallidum* subsp. *pertenue* CDC 2575 antigen, whereas *T. pallidum* subsp. *pertenue* Gauthier contained two reactive polypeptides with apparent molecular sizes of 42 and 40 kDa. We presume that the 40-kDa band is a breakdown product of TmpA resulting from the degradation of the protein occurring during isolation and storage of this particular preparation. Although the mobility by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the TpE antigen from *T. pallidum* subsp. *pertenue* Gauthier is lower than that of *T. pallidum* subsp. *pallidum* Nichols, this difference in apparent molecular mass is not subspecies specific, as TpE from *T. pallidum* subsp. *pertenue* CDC 2575 was identical to that of *T. pallidum* subsp. *pallidum* Nichols.

No discrimination between syphilis sera and yaws sera was possible by analysis of Western blots of total *T. pallidum* subsp. *pallidum* or *T. pallidum* subsp. *pertenue* antigens. Similarly, Baker-Zander and Lukehart (6) detected no differences when *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* immunoblots were incubated with rabbit anti-*T. pallidum* subsp. *pallidum* and anti-*T. pallidum* subsp. *pertenue* antiserum, followed by incubation with ¹²⁵I-labeled protein A. Fohn et al. (11) were unable to demonstrate significant differences between syphilis sera and sera from pinta patients in reactivity to various *T.*

FIG. 4. Reactivity of overlapping nonapeptides of TpF1 and TyF1 with various sera. (A) Sequences of two sets of overlapping nonapeptides based on the amino acid sequences of residues 32 to 48 of TpF1 and TyF1; (B) ELISA results of 8 representative serum samples with the individual nonapeptides. Each bar represents the optical density value of a serum sample with a particular nonpeptide. Graphs: 1 and 2, syphilis sera; 3 and 4, yaws sera; 5, rabbit anti-*T. pallidum* subsp. *pallidum*; 6, rabbit anti-*T. pallidum* subsp. *pertenue*; 7, rabbit anti-PepA; 8, rabbit anti-PepB. Sera from human controls and noninfected rabbits gave results similar to those in panels 1 to 6.

pallidum subsp. *pallidum* protein antigens. Pinta is caused by another member of the *T. pallidum* subsp. *pallidum* group, *Treponema carateum*, and this organism is even more refractory in culturing than is *T. pallidum* subsp. *pallidum* or *T. pallidum* subsp. *pertenue*. Occasional slight quantitative differences in Western blot patterns were noticed, but these may reflect variations in the stage of the disease among patients at the time of collection of sera. Because of the difficulty in obtaining a precise clinical history from the majority of yaws patients, we do not know exactly the period of time between the onset of the disease and the time of sampling of the sera.

At present, the only known molecular difference between a *T. pallidum* subsp. *pallidum* and a *T. pallidum* subsp. *pertenue* strain is the amino acid at position 40 in the 19-kDa polypeptides TpF1 and TyF1 (30). Recently, the DNA sequence of the gene encoding the 19-kDa antigen C1-5 was published (44) and this sequence is identical to that of TpF1 (30). TpF1 and C1-5 are likely to be identical to the well-characterized 4D antigen investigated by Fehniger et al. (10).

The single amino acid difference between TpF1 and TyF1 at position 40 in the polypeptide chain has a remarkable effect on the mobility of the proteinase K-resistant products of these antigens (30). At present, it is not known whether this difference in mobility is due to a difference in folding or to a difference in susceptibility of the polypeptide chain to proteinase K digestion or both.

In this study, we examined whether or not the fragments of 95-kDa TpF1 and 115-kDa TyF1 generated by proteinase K could be used to discriminate syphilis sera from yaws sera. These two antigenic components, however, behaved similarly with respect to antibody binding. Sera from syphilis patients and from yaws patients from Indonesia were on average more reactive to 95-kDa TpF1 and 115-kDa TyF1 compared with yaws sera from patients in Surinam and Ghana. We do not know whether or not this is due to strain differences or to genetic differences between populations. Sera from antibiotic-treated syphilis patients were less reactive than those from nontreated patients, indicating that antibody levels to these antigens drop after treatment.

No correlation was found between the test results of classical syphilis serology and the reactivity of the syphilis sera and yaws sera with 95-kDa TpF1 on the Western blots. Sera with high antibody titers in classical syphilis tests were not always reactive with 95-kDa TpF1 and 115-kDa TyF1.

To further identify a putative subspecies-specific epitope in the region around position 40, we synthesized two sets of peptides based on the TpF1 and the TyF1 sequences. However, no difference was detectable in the reactivity in the ELISA either of polyclonal yaws sera and syphilis sera or of polyclonal anti-peptide rabbit sera. Therefore, it seems unlikely that the single amino acid difference in TpF1 and TyF1 can be used as a means to differentiate syphilis and yaws by serological testing.

Although the TpF1- and TyF1-derived 15-mer peptides, PepA and PepB, did not differ in their reactivity to antibodies in yaws sera or syphilis sera, virtually all sera from nontreated cases of syphilis were reactive with these peptides. This indicates that TpF1-derived peptides are potentially useful reagents for serodiagnostic purposes. Radolf et al. (35) used the nondenatured 4D antigen in an ELISA and showed it to be a sensitive test for the serodiagnosis of syphilis and frambesia. We found that over 50% of the yaws sera were nonreactive with the peptides PepA and PepB, suggesting that the immune response to TpF1 or TyF1 during an infection with *T. pallidum* subsp. *pertenue* tends to differ from that with *T. pallidum* subsp. *pallidum*.

Although differentiation of treponematoses by serology is at present not possible, the small variability in DNA sequences allows strain discrimination. Recently, we determined that the presence of either an A or a G at the position corresponding to the second residue of codon 40 in the DNA encoding TpF1 or TyF1 was also found in the genomic DNA of *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue* (31). This was done by hybridization of labeled oligonucleotides with in vitro-amplified treponemal DNA of various clinical treponemal isolates. These experiments indicate that strains from syphilitic patients generally contain an adenine, whereas yaws strains have a guanine, as the second residue in codon 40 of the genes encoding TpF1 or TyF1.

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