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Cloning and Expression of the Major 47-Kilodalton Surface Immunogen of *Treponema pallidum* in *Escherichia coli*

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Monoclonal antibodies directed against the 47-kilodalton (kDa) major outer membrane surface immunogen of virulent *Treponema pallidum* subsp. *pallidum* were used to select *Escherichia coli* recombinant clones expressing the 47-kDa immunogen. The phenotype of the clones was dependent on the presence of recombinant plasmid in the host cell. Southern hybridization revealed that the cloned *T. pallidum* subsp. *pallidum* DNA sequence was an accurate representation of the *T. pallidum* subsp. *pallidum* genomic DNA arrangement. Purified immunoglobulin G from rabbits experimentally infected with *T. pallidum* subsp. *pallidum* and human secondary syphilitic sera specifically reacted with the clones, while normal human serum or immunoglobulin G from normal rabbit serum did not. Results of Southern hybridization indicated that a homologous 47-kDa immunogen gene was absent in at least four species of nonpathogenic treponemes tested, as well as from total rabbit genomic DNA. Rabbit anti-*T. phagedenis* biotype Reiter (treponemal nonpathogen) antiserum and a monoclonal antibody directed against a common treponemal determinant were unreactive with the clones. Western blotting and radioimmunoprecipitation experiments with specific monoclonal antibodies revealed that the recombinant (*E. coli*) and native (*T. pallidum* subsp. *pallidum*) forms of the antigen had identical electrophoretic mobilities. The availability of recombinant 47-kDa immunogen provides a new opportunity for biochemical analysis of the protein, structure-function studies, examination of its role in microbial pathogenesis, and assessment of its diagnostic and vaccinogenic potentials.

Syphilis is a chronic, complex, sexually transmitted disease of humans caused by the bacterium *Treponema pallidum* subsp. *pallidum* (30). Because *T. pallidum* subsp. *pallidum* cannot be readily cultured in vitro, in vivo culture in rabbits and hamsters has provided the practical means of obtaining bacteria for experimentation (30). Only limited quantities of bacteria, however, can be obtained from in vivo cultivation. Also, *T. pallidum* subsp. *pallidum* isolated from tissue characteristically is contaminated with host tissue components, regardless of the bacterial isolation strategy used. Furthermore, isolation and purification processes compromise the structural integrity of the fragile *T. pallidum* subsp. *pallidum* organisms. These limitations have restricted progress in elucidating the morphology, structure, physiology, and genetics of *T. pallidum* subsp. *pallidum* (30). Perhaps most importantly, they have hampered the identification of molecular components of the organism that may influence pathogenesis and the immune response of the host during syphilitic infection. With one exception (7), it has not been feasible to directly assess the biological importance of individual antigens of *T. pallidum* subsp. *pallidum*.

Despite these obstacles, potentially important antigens or immunogens of *T. pallidum* subsp. *pallidum* have been identified in several laboratories (1–4, 8, 13, 15–19, 21, 26, 27, 33, 36–38). Potential biological significance was previously assigned to a major immunogenic surface antigen of *T. pallidum* subsp. *pallidum* having a molecular mass of 47 kilodaltons (kDa) (13, 18). Biologically active monoclonal antibodies (MAbs), in combination with various in vitro and in vivo assays, were used to characterize the immunogen (13, 18, 19, 25). The 47-kDa antigen was shown to be (i) surface associated (13, 18); (ii) abundant (13, 18, 19); (iii) highly immunogenic in both rabbits and humans (2, 8, 13, 16, 33, 38); (iv) proteinaceous (13); (v) present in at least three subspecies of pathogenic *T. pallidum*, the etiological agents of venereal syphilis, endemic syphilis, and yaws (13, 18, 19); and (vi) absent in nonpathogenic, saprophytic treponemes (13, 18, 19, 25). Anti-47-kDa MAbs possess diagnostic value (19, 25); they bind strongly in immunofluorescence assays to *T. pallidum* subsp. *pallidum* isolated from human syphilitic lesions (19). Anti-47-kDa MAbs also bind to *T. pallidum* subsp. *pallidum* in the *T. pallidum* immobilization assay, resulting in complement-dependent immobilization of motile organisms; these MAbs in the presence of complement neutralize (kill) *T. pallidum* subsp. *pallidum* in the in vitro-in vivo neutralization test of Bishop and Miller (see references 5, 13, and 19).

To date, the 47-kDa immunogen remains a primary candidate with diagnostic and vaccinogenic potential. In this paper, we describe the cloning and expression of the *T. pallidum* subsp. *pallidum* 47-kDa immunogen gene in *Escherichia coli*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and DNAs. The virulent Nichols strain of *T. pallidum* subsp. *pallidum* was used as the representative pathogen in this study. It was maintained and cultivated in the testicles of New Zealand White rabbits as previously described (24, 28). Nonpathogenic strains of treponemes *T. phagedenis* biotype Reiter, *T. denticola*, *T. scoliodontum*, and *T. vincentii* were cultivated in vitro and were provided to us (34). *Escherichia coli* RR1 was used as the recipient host strain for cloning and transformation experiments (34). A summary of the relevant recombinant plasmids used in this study is given in Table 1. *E. coli* RR1 harboring the plasmid pMN7 (old designation, RICB2-1) has been described previously (24); pMN7 originated from the

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TABLE 1. Characteristics of recombinant plasmids

Plasmid (strain)	T. pallidum subsp. pallidum DNA insert (kb)	Immunogen expressed (kDa)	Relevant MAb or polyclonal antibody reactivity	Reference
pBR322	None	None	None	24
pMN7 (RICB2-1)	3.7	44[a]	IRS[b]	24
pMN20	1.75	34	3B5, 9B12, 10G2	34
pMN23	5.4	47	IIE3, 8G2	This report
pMN24	5.4	47	IIE3, 8G2	This report

[a] Unrelated to the 47-kDa antigen of clones pMN23 and pMN24, as shown by MAb (24) and genetic studies (unpublished data).

[b] Immune rabbit serum obtained from T. pallidum subsp. pallidum -infected rabbits.

clone bank referenced below and contains a 3.7-kilobase (kb) DNA insert of T. pallidum subsp. pallidum DNA cloned by G · C tailing into the PstI site of pBR322. It encodes a 44-kDa recombinant antigen unrelated to the 47-kDa antigen (unpublished genetic data). The 44-kDa antigen of clone pMN7 is immunoprecipitable by immune rabbit serum (24) but not by any of our current MAbs. Clone pMN20, encoding and expressing a 34-kDa surface antigen of T. pallidum subsp. pallidum, has been described previously (34). All plasmid derivatives were propagated in E. coli RR1 and isolated and purified by the method of Norgard (22). New Zealand White normal male rabbit liver DNA and treponemal DNAs were isolated as previously described (23).

Cloning procedures and restriction enzyme analyses. A preestablished pBR322 hybrid plasmid clone bank was used as the potential source of antigen-expressing clones (24). The bank was constructed by using combined *Alu*I and *Hae*III partial restriction enzyme digests of T. pallidum subsp. pallidum DNA which was subsequently tailed with deoxycytidine residues and cloned into pBR322 (deoxyguanosine tailed at the PstI site to regenerate PstI sites flanking the cloned inserts) (24). Restriction enzymes for the analysis of plasmids and digestion of genomic DNA were purchased from New England BioLabs, Inc., Beverly, Mass.

Radiolabeling of bacteria, DNA, and proteins. T. pallidum subsp. pallidum was purified from rabbit testicular tissue by the Percoll density gradient method (9); bacteria were radiolabeled with $Na^{125}I$ by a lactoperoxidase method (1, 13). Strains of E. coli RR1 harboring various pBR322 plasmid derivatives were metabolically labeled with L-[^{35}S]methionine as previously described (24). All probe DNA was radiolabeled with [α-^{32}P]dCTP by using the nick-translation kit from New England Nuclear Corp., Boston, Mass. Antibody probes were radiolabeled with $Na^{125}I$ by a chloramine T method (12).

Southern gel hybridizations. Southern blots were performed by the previously published method (23, 31, 34). For one probe, a DNA restriction enzyme fragment was isolated from low-melt agarose by Elutip (Schleicher & Schuell, Inc., Keene, N.H.) affinity chromatography (29). Hybridization with DNA probes was carried out in 2× Denhardt solution (31) containing 6× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0]), 1 mM EDTA, and 100 μg of sheared, denatured salmon sperm DNA per ml (31). After hybridization for 16 h at 68°C, filters were washed three times (30 min per wash) at room temperature with 500-ml portions of 2× SSC–0.1% sodium dodecyl sulfate and then three times at room temperature (30 min per wash) with 500-ml portions of 0.1× SSC–0.1% sodium dodecyl sulfate. Filters were dried and subjected to autoradiography (14).

MAbs, antisera, and antibody probes. Murine MAbs 8G2 (immunoglobulin G1 [IgG1]), 11E3 (IgG2a), and 3B5 (IgG1) directed specifically against T. pallidum were generated, maintained, and characterized as previously described (13, 18, 19, 25, 28, 34). MAb 11E3 has previously been described in detail (13, 18, 19, 25). MAbs 8G2 and 11E3 are directed against the major 47-kDa immunogen (13, 18). MAb 3B5 reacts with a 34-kDa surface immunogen of T. pallidum (34). MAbs were used within in vitro hybridoma clone supernatants or were affinity purified from hybridoma clone supernatants on individual protein A-Sepharose columns (6). MAbs C2-1 (IgM) and H9-1 (IgG1), directed against common and pathogen-specific epitopes of T. pallidum, respectively (11, 17), were a gift of Sheila A. Lukehart. Normal rabbit serum was collected from Venereal Disease Research Laboratory nonreactive New Zealand White male rabbits. Rabbit anti-T. pallidum serum (immune rabbit serum) was obtained and pooled from four animals 3 to 12 months following a firm orchitis in both testicles after T. pallidum intratesticular infection; immune rabbits were shown to be chancre immune when challenged intradermally with 10^5 motile treponemes per site (13). Rabbit anti-T. phagedenis biotype Reiter antiserum was provided by S. A. Lukehart. Seven human secondary syphilitic serum samples were provided by George Wendel; these sera were collected for routine serological diagnostic confirmation (within the past year) from females with confirmed secondary syphilis. Excess serum from each patient, which was stored at −70°C and used in this study, was obtained from the clinical laboratory in lieu of discard by the laboratory. IgG was isolated from sera by sodium sulfate precipitation and DEAE-cellulose column chromatography (34). Affinity-purified rabbit anti-mouse IgG, goat anti-rabbit IgG (both were heavy- and light-chain specific), and goat anti-mouse IgM (μ-chain specific) were supplied by Ellen Vitetta.

RICB assay. The radioimmuno-colony blot (RICB) assay for the detection of E. coli clone colonies synthesizing T. pallidum antigens was carried out by the method of Norgard and Miller (24) with minor modifications (34).

RIP. Radioimmunoprecipitation (RIP) was performed as previously described (34), with minor modifications. For the RIP of T. pallidum subsp. pallidum antigens, approximately 5×10^6 cpm of ^{125}I-labeled treponemes (1 to 3 cpm per treponeme) was incubated in 1.0 ml of solubilization buffer (10 mM Tris hydrochloride [pH 7.8], 150 mM NaCl, 10 mM EDTA, 0.2% Zwittergent 3-12 [Calbiochem-Behring, La Jolla, Calif.]). For [^{35}S]methionine-labeled E. coli, about 7×10^7 cpm was used. Goat anti-mouse IgG (10 μg) was added after the primary MAb (30 min at 4°C with agitation) as a bridge for IgG1 MAbs 8G2 and 3B5. Solubilized immunoprecipitates were ultimately subjected to sodium dodecyl sulfate-polyacrylamide gel (10%) electrophoresis after reduction at 100°C in 5% 2-mercaptoethanol (34). ^{14}C-labeled molecular weight markers were used as previously described (34). Different radiolabeled compounds (^{35}S, ^{14}C, and ^{125}I) were detected on the same gel after treatment with En-^{3}Hance (New England Nuclear) for ^{35}S and ^{14}C, followed by autoradiography (14).

Western blots. Western blots were performed as previously described (18). For the analysis of T. pallidum antigens, approximately 10^7 solubilized treponemes per polyacrylamide gel lane were blotted. For E. coli recombinant derivatives, approximately 10^8 solubilized E. coli cells per gel lane were used.

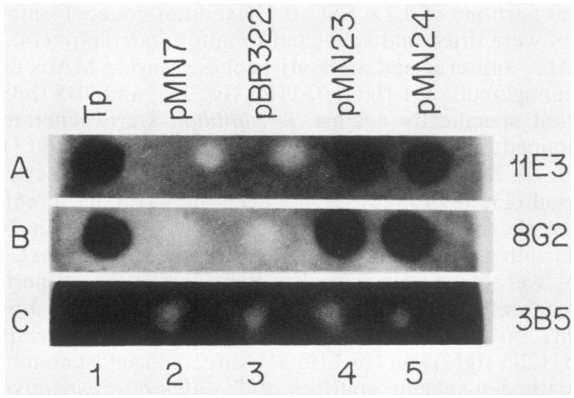

FIG. 1. RICB assay of *E. coli* recombinant DNA clone colonies expressing the 47-kDa surface immunogen of *T. pallidum*. *T. pallidum* subsp. *pallidum* cells (lane 1) were spotted onto each filter as a positive control. Negative control clones included pMN7 (lane 2) and pBR322 in *E. coli* RR1 (lane 3). Filters were reacted with MAbs 11E3 (row A), 8G2 (row B), and 3B5 (row C) (negative control), prior to probing with ^{125}I-labeled rabbit anti-mouse IgG.

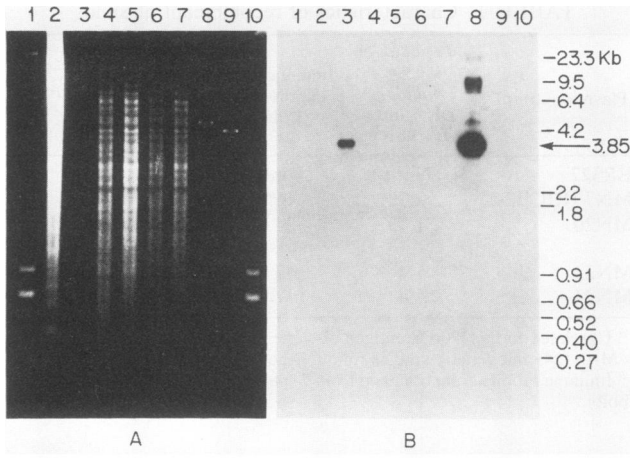

FIG. 3. Southern blot hybridization of *Hin*dIII-restricted genomic DNAs and recombinant plasmid pMN23 encoding the 47-kDa immunogen of *T. pallidum*. (A) Agarose gel (1%) containing *Hin*dIII-restricted genomic DNA of rabbit (lane 2), *T. pallidum* subsp. *pallidum* (lane 3), *T. denticola* (lane 4), *T. phagedenis* (lane 5), *T. scoliodontum* (lane 6), *T. vincentii* (lane 7), recombinant plasmid pMN23 (lane 8), and plasmid pBR322 (lane 9). Lanes 1 and 10 contain combined pBR322-*Alu*I and bacteriophage lambda-*Hin*dIII molecular weight markers. (B) Southern blot of panel A; a purified 3.85-kb *Hin*dIII fragment of plasmid DNA from clone pMN23 was used as the labeled hybridization probe. Note the presence of the 3.85-kb homologous DNA *Hin*dIII-fragment present only in *T. pallidum* genomic DNA (lane 3) and the recombinant plasmid pMN23 (lane 8).

RESULTS

Identification of antigen-expressing clones. Figure 1 shows the results of RICB assays with MAbs. Two recombinant DNA clones, pMN23 and pMN24, were isolated which reacted with anti-47-kDa MAbs 11E3 and 8G2 but not with the negative control MAb 3B5. All three MAbs failed to react with the negative control clone pMN7 or pBR322. MAb 3B5, directed against a 34-kDa surface immunogen of *T. pallidum* (34), reacted only with *T. pallidum* subsp. *pallidum*.

Immune rabbit serum IgG reacted in the RICB assay with clones pMN23, pMN24, and pMN7, as expected, while IgG from normal rabbit serum did not (not shown). Rabbit anti-*T. phagedenis* biotype Reiter antiserum, possessing antibodies against common treponemal determinants, also failed to react with clones pMN23 and pMN24 (not shown). MAb H9-1, directed specifically against the 47-kDa immunogen (11, 17), reacted strongly with clones pMN23 and pMN24 but not with pMN7 or pBR322 (not shown). In additional RICB assays, MAb C2-1, directed against a common treponemal epitope (11, 17), reacted with *T. pallidum* subsp. *pallidum* and *T. phagedenis* biotype Reiter but not with any *E. coli* clones (not shown).

Further support for the plasmid-encoded, antigen-expressing phenotype was demonstrated by the fact that purified plasmid DNA from RICB-positive recombinant clones was capable of transforming the 47-kDa antigen-expressing phenotype to normal *E. coli* host cells at a frequency of 100% (200 of 200 random transformants tested).

Restriction enzyme map of the 47-kDa immunogen-expressing clone pMN23. Figure 2 shows a preliminary restriction enzyme map of the 5.4-kb insert of plasmid pMN23. The pMN23 insert is flanked by short deoxycytidine-deoxyguanosine tails inside *Pst*I sites, with five internal *Pst*I sites located within the insert. A strategic fragment for structural analysis of the 47-kDa immunogen-encoding region included a 3.85-kb *Hin*dIII fragment; subcloning of this fragment into the *Hin*dIII site of pBR322, however, failed to result in expression of the relevant antigen's epitope(s) when 54 ampicillin-resistant, tetracycline-sensitive 3.85-kb *Hin*dIII fragment-containing subclones were tested in the RICB assay with MAb 11E3 or 8G2. Analogous restriction enzyme mapping of clone pMN24 revealed the presence of similar restriction enzyme fragments as those of pMN23. pMN23 and pMN24 may be identical; final conclusion awaits more rigorous analysis.

Specificity of the cloned 47-kDa immunogen gene sequence among *T. pallidum* subsp. *pallidum* DNA. Owing to the potential presence of contaminating rabbit host DNA in *T. pallidum* subsp. *pallidum* DNA preparations used as a source of DNA for cloning, it was essential to establish the *T. pallidum* origin of the cloned DNA sequence. It also was

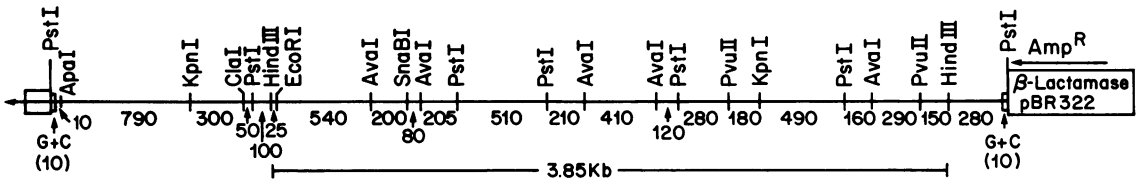

FIG. 2. Partial restriction enzyme map of the 5.4-kb insert of pMN23 encoding the 47-kDa immunogen of *T. pallidum*. The *Eco*RI site of pBR322 is located to the right of the figure. Arrows indicate the normal direction of transcription for the beta-lactamase gene of pBR322. The insert is flanked by *Pst*I sites just outside short G · C tails and possesses a 3.85-kb internal *Hin*dIII fragment.

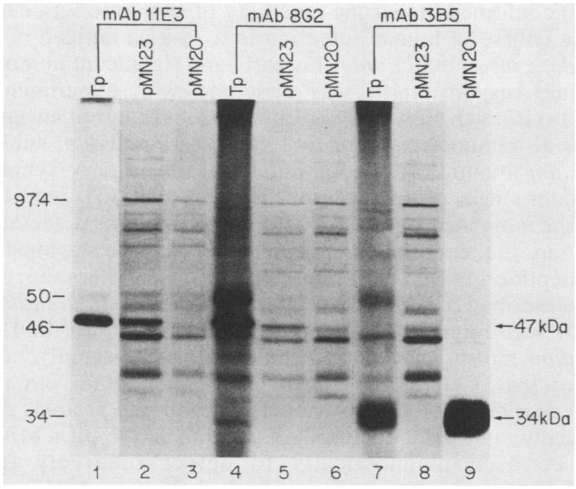

FIG. 4. RIP of the 47-kDa immunogen of *T. pallidum* from ^{125}I-labeled *T. pallidum* subsp. *pallidum* and ^{35}S-labeled *E. coli* recombinant clone pMN23. Lanes 1 to 3, 4 to 6, and 7 to 9 were immunoprecipitated with MAbs 11E3, 8G2, and 3B5 (control), respectively. Lanes 1, 4, and 7 contained ^{125}I-labeled *T. pallidum* antigens. Lanes 2, 5, and 8 contained ^{35}S-labeled products from the 47-kDa immunogen-expressing clone pMN23. Lanes 3, 6, and 9 contained ^{35}S-labeled products from clone pMN20, which expresses a 34-kDa immunogen of *T. pallidum* recognized by MAb 3B5. Protein molecular masses are indicated on the left (in kilodaltons), derived from ^{14}C-molecular weight markers (34) electrophoresed on the gel. Arrows indicate the location of the 47- and 34-kDa immunogens.

important to determine whether homologous gene sequences existed among immunologically related nonpathogenic treponemes. To address these possibilities, the 3.85-kb *Hin*dIII fragment of pMN23 was isolated, labeled, and used as a hybridization probe in Southern blot analysis (Fig. 3B). The agarose gel (1%) (Fig. 3A) contained *Hin*dIII-restricted preparations of genomic DNAs from *T. pallidum* subsp. *pallidum*, rabbit liver, and four nonpathogenic treponemes. Figure 3A, lanes 2 and 4 through 7, containing other than *T. pallidum* DNA, were overloaded to ensure a conclusive result. In Fig. 3B, lanes 3 and 8, the 3.85-kb *Hin*dIII probe hybridized to a 3.85-kb *Hin*dIII fragment of *T. pallidum* subsp. *pallidum* DNA and to itself; no hybridization with rabbit DNA, the DNAs of four nonpathogenic treponemes, or control DNA (lambda or pBR322) was observed. Multiple hybridizing bands observed in *Hin*dIII-cleaved pMN23 (Fig. 3B, lane 8) represented the minute proportion of pMN23 not completely restricted by *Hin*dIII treatment.

When Southern blots identical to Fig. 3B were probed and washed less extensively to the point at which nonhomologous DNA-DNA hybridization could be observed to occur with either lambda DNA or pBR322 DNA, no hybridization of the 3.85-kb *Hin*dIII fragment probe with any *Hin*dIII DNA fragment of the nonpathogenic treponemes was observed (not shown). Intact plasmid pMN23 (containing the entire 5.4-kb sequence), used as a labeled probe under reduced stringency, also did not hybridize to any *Hin*dIII DNA fragment of the nonpathogens (not shown). The inability to detect a homologous 47-kDa immunogen gene sequence in the nonpathogenic treponemes therefore did not appear to be the result of excessively stringent hybridization conditions used in the Southern blot.

When Southern gel blots identical to Fig. 3B were probed with labeled pBR322 (vector DNA), no hybridization of a 3.85-kb *Hin*dIII fragment with *T. pallidum* subsp. *pallidum* DNA or with the 3.85-kb *Hin*dIII fragment of clone pMN23 was observed (data not shown). In contrast, intact labeled pMN23 plasmid DNA used as a hybridization probe hybridized to the corresponding *T. pallidum* subsp. *pallidum* DNA sequences in both *T. pallidum* subsp. *pallidum* genomic DNA and clone pMN23, as well as to the pBR322 DNA sequences, but not to rabbit DNA or the genomic DNAs of the nonpathogenic treponemes, as predicted (not shown).

Expression of the 47-kDa immunogen in *T. pallidum* and *E. coli*. Figure 4 shows the results of RIP assays performed with ^{125}I-labeled *T. pallidum* subsp. *pallidum* and ^{35}S-labeled recombinant clones pMN23 and pMN20 as antigens. Solubilized antigens were immunoprecipitated with MAbs 11E3, 8G2, and 3B5. MAb 3B5, directed against a 34-kDa immunogen of *T. pallidum* (lane 7), was used as a control. MAbs 11E3 and 8G2 immunoprecipitated the 47-kDa immunogen from ^{125}I-labeled *T. pallidum* subsp. *pallidum* (Fig. 4, lanes 1 and 4). An antigen with an apparently identical M_r to the 47-kDa immunogen of *T. pallidum* subsp. *pallidum* was immunoprecipitated by MAbs 11E3 and 8G2 from clone pMN23 (lanes 2 and 5) but not from *E. coli* harboring the control hybrid plasmid pMN20 (lanes 3 and 6), which encodes a 34-kDa *T. pallidum* antigen (lane 9). In lanes 1, 4, and 7, the extraneous band at molecular mass 50 kDa represents rabbit host heavy-chain immunoglobulin which copurifies with *T. pallidum* and which is labeled by lactoperoxidase-catalyzed iodination (19); other irrelevant bands in lanes 1 through 9 are due to nonspecific absorption of labeled products to *Staphylococcus aureus* cells.

An analogous result to that of the RIP assay was obtained by using Western blotting (Fig. 5). Anti-47-kDa MAb 8G2 reacted with 47-kDa antigens from *T. pallidum* subsp. *pallidum*, clone pMN23, and clone pMN24 but not with any negative control clones pMN20, pBR322, or pMN7 (Fig. 5). A similar Western blot probed with anti-47-kDa MAb 11E3

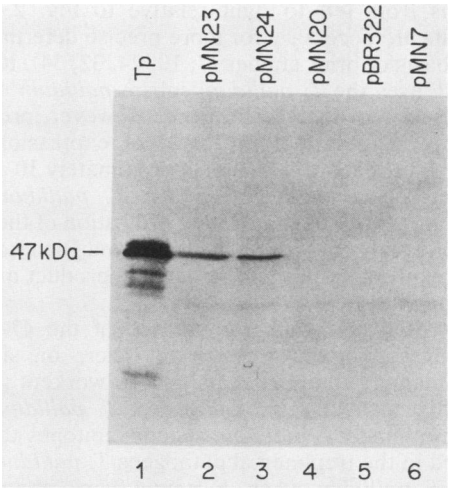

FIG. 5. Western blot of the 47-kDa immunogen expressed by recombinant clones pMN23 and pMN24. Solubilized antigens were detected after gel electrophoresis and protein transfer by incubation with anti-47-kDa MAb 8G2, which produced its characteristic reactivity profile with the 47-kDa *T. pallidum* immunogen (18, 25) (lane 1). Lanes 2 and 3 contained the 47-kDa immunogen-expressing clones pMN23 and pMN24, respectively. Lanes 4, 5, and 6 contained the 34-kDa immunogen-expressing clone pMN20, pBR322 in *E. coli* RR1, and the 44-kDa immunogen-expressing clone pMN7, respectively, as negative controls.

gave identical results (not shown). When another blot was probed with MAb 3B5, it revealed the presence of the 34-kDa antigen in *T. pallidum* and clone pMN20 but not in clones pMN23, pMN24, pMN7, or pBR322, as expected (not shown).

Further Western blotting experiments revealed that at least six of seven human secondary syphilitic sera reacted with the recombinant form of the 47-kDa immunogen (one result was equivocal), while normal human serum did not (not shown). The same human syphilitic sera were unreactive with *E. coli* harboring the cloning vector alone. The experiments confirmed the reactivity of human antibodies elicited in response to the naturally acquired infection by *T. pallidum* subsp. *pallidum* with the recombinant DNA-derived 47-kDa immunogen expressed in *E. coli*.

DISCUSSION

Evidence is presented for the cloning and expression of the major 47-kDa surface immunogen of *T. pallidum* subsp. *pallidum* in *E. coli*. The 5.4-kb DNA insert encoding the 47-kDa immunogen possessed ample coding capacity for the immunogen; approximately 1.3 kb of DNA would be required to encode a mature 47-kDa antigen. A 3.85-kb *Hin*dIII DNA fragment subclone of the 5.4-kb total DNA insert was incapable of expressing the relevant antigen's epitope(s) when subclones were analyzed with MAb 11E3 or 8G2. Additional preliminary subcloning experiments suggest that the rightward three *Pst*I fragments of clone pMN23 (Fig. 2) are not necessary for expression of the 47-kDa immunogen (unpublished data). Portions of all three of the leftward *Pst*I fragments of pMN23 appear to be required for expression of the 47-kDa gene product, but apparently only a small rightward portion of the left 1,150-base-pair *Pst*I fragment is required for expression. Deletion of the 510-base-pair *Pst*I fragment results in a truncated gene product with a molecular mass of about 44 to 45 kDa (reactive with MAb 11E3). These preliminary results suggest that the direction of transcription is from left to right relative to Fig. 2. Further experiments are necessary for more precise determinations.

On the basis of prior studies (7, 10, 24, 32, 34), it is likely that *E. coli* uses the *T. pallidum* subsp. *pallidum* promoter for transcription of the 47-kDa gene. However, preliminary experiments suggest that the level of expression of the 47-kDa gene product in *E. coli* is approximately 10^{-3} to 10^{-4} as efficient (on a per-cell basis) as *T. pallidum* subsp. *pallidum* (unpublished data). Initial utilization of the expression vector system described by Tabor and Richardson (35) increased expression of the 47-kDa gene product more than 100-fold (unpublished).

The potential biological significance of the 47-kDa immunogen is supported by work of others on similar or identical immunogens. Lukehart and co-workers (3, 4, 15) reported that a 48-kDa immunogen of *T. pallidum* subsp. *pallidum* contained *T. pallidum*-specific epitopes that could be detected in the treponemal pathogens *T. pallidum* subsp. *pallidum*, *T. pallidum* subsp. *pertenue* (yaws organism), *T. paraluis-cuniculi* (agent of rabbit venereal spirochaetosis), and *T. hyodysenteriae* (agent of swine dysentery). Lukehart also observed an early and significant humoral response to the 47-kDa immunogen of *T. pallidum* subsp. *pallidum* in patients infected with *T. pallidum* subsp. *careteum* (pinta organism) (S. A. Lukehart, personal communication). Hanff et al. (8) showed an early humoral immune response during human syphilis to *T. pallidum* subsp. *pallidum* antigens having molecular masses of 45 to 47 kDa. Baker-Zander et al. (2) confirmed the strong reactivity of syphilitic sera early in the course of human infection to a 48-kDa antigen of *T. pallidum* subsp. *pallidum*. This early and significant humoral immune response also was observed with experimental rabbits (16). van Eijk and van Embden (38) reported an early humoral immune response to a 46-kDa *T. pallidum* subsp. *pallidum* immunogen among humans with primary syphilis and later stages of the disease. Strugnell et al. (33) reported that the most vigorous humoral immune response detectable early in the experimental rabbit was directed against a polypeptide of 47 kDa. Thornburg and co-workers (36, 37) also described a major 45-kDa immunogen of *T. pallidum*, which was found in *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*. Penn et al. (27) recently concluded that *T. pallidum* subsp. *pallidum* contains an immunodominant, 47-kDa major outer membrane protein. Additionally, our earlier findings (19, 25) that anti-47-kDa MAbs can be used diagnostically to detect relatively few treponemes has been confirmed by Lukehart et al. (17) and Hook et al. (11). Lukehart et al. (16) also reported a correlation between early immune clearance of infecting *T. pallidum* and healing of the primary lesion in the experimental rabbit; it was postulated that primary lesion healing may be influenced by antibody directed against immunodominant molecules, such as the 47-kDa immunogen (16).

Evidence for the potential biological importance of the 47-kDa immunogen, although compelling, essentially has been indirect. The availability of purified 47-kDa recombinant immunogen provides an opportunity for direct diagnostic, pathogenesis, and vaccine assessment. That both the native and recombinant DNA-derived forms of the 47-kDa immunogen possess identical electrophoretic mobilities on polyacrylamide gels and that antibodies present in human syphilitic sera bind to the recombinant form of the antigen suggest that the recombinant molecule may be used in place of the native immunogen in these studies.

With serum from *T. pallidum*-infected rabbits or murine MAbs directed against the 47-kDa immunogen of *T. pallidum*, it was previously reported that the 47-kDa immunogen was pathogen specific (13, 18, 19, 25); we did not detect the presence of an analogous 47-kDa antigen in the nonpathogenic treponemes *T. phagedenis* biotype Reiter, *T. denticola*, *T. scoliodontum*, *T. vincentii*, or *T. refringens* when using immune rabbit serum or MAbs directed against the 47-kDa immunogen. Lukehart et al. (15, 17) postulated that a 48-kDa *T. pallidum* immunogen may possess pathogen-specific as well as common treponemal determinants or that common and pathogen-specific determinants may be located on separate polypeptides which comigrate in polyacrylamide gels. When either a 3.85-kb *Hin*dIII DNA fragment of the 5.4-kb encoding sequence or intact hybrid plasmid DNA (containing the entire 5.4-kb encoding sequence) were used as DNA hybridization probes under moderate or low DNA hybridization stringency, no hybridization with any homologous DNA fragment of the nonpathogenic treponemes was observed. Furthermore, MAb C2-1 (from S. A. Lukehart), directed against a common treponemal epitope of a 47-kDa immunogen (11, 17), failed to react with the 47-kDa immunogen-expressing clones, while the pathogen-specific MAb H9-1 (11, 17) reacted with the clones. Thus, genetic and immunologic data provided here support the existence of a pathogen-specific 47-kDa immunogen.

Expression of the 47-kDa immunogen in *E. coli* should allow more precise biochemical analysis of the 47-kDa protein. Lukehart et al. proposed that the 48-kDa im-

munogen of *T. pallidum* may be a glycoprotein (15). Although F-pilin of *E. coli* may be considered a glycoprotein (containing one glucose and possibly one each of galactose and of a dideoxy hexose) (39), the precedent for glycoproteins in procaryotes is poor. The fact that *E. coli* expresses a 47-kDa antigen with an identical electrophoretic mobility to the native 47-kDa immunogen of *T. pallidum* suggests that the 47-kDa immunogen is not a glycoprotein.

The cloning and expression of the 47-kDa immunogen of *T. pallidum* in *E. coli* provide tools to help assess the chemical composition of the protein and the structure-function relationship of the native 47-kDa immunogen in *T. pallidum*, possibly leading to an increased understanding of the biology of this elusive pathogen. DNA encoding the 47-kDa antigen may be useful as a diagnostic DNA probe (20) to identify treponemal pathogens in genital ulcers, skin lesions, and other body fluids. Purified 47-kDa immunogen can be used to reexamine, by in vitro and in vivo methods, both humoral and cell-mediated immune responses to purified antigens; this may help to further clarify the respective roles of both arms of the immune response to *T. pallidum* subsp. *pallidum* infection in the host. The recombinant DNA-derived immunogen also may provide the basis for an improved serological test for syphilis, potentially possessing increased specificity and simplicity over currently used methods. Additionally, the availability of recombinant immunogen should allow direct assessment of its vaccinogenic potential.

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