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Identification, Localization, and Distribution of the PilT Protein in *Neisseria gonorrhoeae*

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A monoclonal antibody (MAb) directed against a highly conserved protein of *Neisseria gonorrhoeae* with a molecular size of 40 kDa was isolated and characterized. The protein antigen detected by this MAb was detected by enzyme-linked immunosorbent assay and immunoblotting in all strains of *N. gonorrhoeae* tested across a wide range of serovars. The 40-kDa protein was found to be expressed at relatively low levels and localized to both the cytosolic and cytoplasmic membrane fractions. Screening of a λgt11 expression library derived from gonococcal genomic DNA with the anti-40-kDa MAb and DNA sequence analysis suggested that the 40-kDa protein and the product of the gonococcal *pilT* gene were identical. Immunoblotting analysis of gonococcal mutants carrying defined mutations in the *pilT* gene confirmed that the 40-kDa protein was indeed PilT. The N-terminal sequence derived by microsequencing of the protein purified from gonococci led to the correction of the previously published *pilT* gene sequence. Sequencing of the *pilT* gene from three different strains revealed an extremely high degree of conservation at both the amino acid and DNA levels.

Neisseria gonorrhoeae infects only humans and leads to a wide spectrum of clinical manifestations. The interaction of gonococci with human cells is mediated by surface components, including pili (34), opacity-associated outer membrane proteins (9), and lipooligosaccharide (20). Many investigations aimed at understanding the pathogenic mechanisms of gonococcal infection have been directed toward understanding the structure and function of these surface-localized components and the regulation of their expression.

The pili of *N. gonorrhoeae* are hairlike filamentous appendages about 7 nm in diameter and up to 2.5 μm in length that appear to play an essential role in the ability of the bacterium to colonize its human host. They consist of a protein subunit of approximately 160 amino acids (30), called pilin, which is encoded by the chromosomal gene *pilE* (21). The short leader sequence and proximal 30 amino acids of gonococcal prepilin show a high degree of homology with prepilins of other gram-negative human pathogens, including *Neisseria meningitidis* (25), *Pseudomonas aeruginosa* (32), *Vibrio cholerae* (31), and certain strains of enteropathogenic *Escherichia coli* (11). Expression of these proteinaceous appendages, which are collectively termed type IV fimbriae (24), is strongly correlated with the ability of the bacterium to colonize the human host. Type IV pilus expression can be associated with other phenotypes, including bacterial growth patterns and a phenomenon termed twitching motility. This latter property is manifested as darting, intermittent translocation of cells in suspension and a spreading colony phenotype (14). Bradley described mutants of *P. aeruginosa* that were hyperpiliated and resistant to pilus-specific phages and that failed to display twitching motility. On the basis of electron microscopic studies, it was concluded that twitching was the result of pilus retraction and that these mutants were deficient in that activity (3). Whitchurch et al. used phenotypic complementation to identify a gene, *pilT*, which simultaneously restored twitching motility, phage sensitivity, and spreading colony morphology to the mutants (36). Gonococci share the pilus-associated properties of twitching motility and distinctive colony morphology (14), and recently, a gonococcal gene bearing striking sequence identity with the *P. aeruginosa pilT* gene was characterized (18). The putative products of both of the *pilT* genes show significant sequence homology with proteins involved in protein export pathways, such as the *Klebsiella oxytoca* PulE protein (7) and the *Xanthomonas campestris* XpsE protein (10). Other members of this expanding family of proteins with consensus nucleotide-binding domains have been implicated in the extracellular localization of toxins and hydrolases by many pathogenic gram-negative bacteria, including cholera toxin (28), pertussis toxin (35), aerolysin (15), and exotoxin A (33). A related protein is also involved in competence for DNA transformation in *Bacillus subtilis* (1).

We report the production and characterization of a monoclonal antibody (MAb), 13C5, directed against the PilT protein of *N. gonorrhoeae*. The availability of this reagent has made it possible to identify and characterize the PilT protein in gonococci, examine its distribution and expression in a large number of strains, and determine its subcellular localization. In the course of these studies, we also found that the original characterization of the *pilT* gene was in error owing to a DNA rearrangement arising during propagation in *E. coli* and here rectify the sequence data.

MATERIALS AND METHODS

Bacterials strains and growth conditions. The various strains used herein were obtained from the American Type Culture Collection (ATCC), Rockville, Md., the Laboratoire de Santé Publique du Québec (Québec, Canada), and the Laboratory of Microbiology of the Centre Hospitalier de l'Université Laval (Québec, Canada). Strain 2170 has been described previously (4). Gonococci were grown on GC medium agar base with 1% IsoVitaleX or in liquid medium in the

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presence of 5% CO_2. *E. coli* Y 1090 (Promega Biotech) was used for λgt11 phage propagation. Recombinant plasmids were constructed with the cloning vector pBluescript KS+ (Stratagene). *E. coli* strains were grown at 37°C in Luria-Bertani medium supplemented with appropriate antibiotics. Plasmid clone p2A2 and lambda phage clone 18/4 carrying the *pilT* locus of *N. gonorrhoeae* MS11 have been described previously (18).

Production and characterization of anti-40-kDa antibody. Antibody-producing cells were obtained by fusion of spleen cells from BALB/c mice, immunized with whole bacteria, and myeloma cell lines (SP2/O-Ag14) in the presence of polyethylene glycol as described previously (4). The antibody-producing hybridomas were selected by enzyme-linked immunosorbent assay (ELISA) using microtiter plates coated with whole bacteria (10^7 bacteria per well) (5) or by Western blotting (immunoblotting). Antibody isotypes were determined with a commercial kit, the Immunoselect Isotyping System (Gibco BRL, Burlington, Ontario, Canada). The anti-40-kDa MAb 13C5 was purified on a protein G column as described previously (4).

Protein gel electrophoresis and immunoblotting. Samples were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis by mixing them with an equal volume of sample buffer and boiling the mixture for 3 min. SDS-PAGE was performed as described by Laemmli (17). Two-dimensional gels were prepared for nonequilibrium pH gel electrophoresis by using ampholytes at pH 3 to 10 in the first dimension and SDS-PAGE in the second dimension as described previously (23). After electrophoresis, gels were fixed and stained with Coomassie blue. For immunoblotting, proteins from SDS-PAGE gels were transferred electrophoretically to nitrocellulose sheets and incubated with MAb 13C5 and then with ^{125}I-labelled anti-mouse antiserum as described previously (4).

Localization of the 40-kDa protein. Bacteria were collected by centrifugation, and membranes were purified as described previously (22). Briefly, after lysis by sonication in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.4)–phenylmethylsulfonyl fluoride and centrifugation at 12,000 × *g*, the membrane fraction was collected by centrifugation of the supernatant at 100,000 × *g* for 1 h and the pellet was suspended in phosphate-buffered saline. The presence of 40-kDa protein in the soluble fraction (periplasm and cytoplasm) and in the insoluble fraction (total membranes) was analyzed by immunoblotting using MAb 13C5. Finally, the cytoplasmic and outer membranes were separated by treatment of the pellet with 0.5% Sarkosyl. The samples were centrifuged at 100,000 × *g* for 1 h; the supernatant fraction contained solubilized cytoplasmic membrane proteins, while outer membrane proteins were found in the pellet.

Sample preparation for protein microsequencing. Sarkosyl-soluble proteins derived from total membrane preparations were subjected to two-dimensional gel electrophoresis. Following transfer to polyvinylidene difluoride Immobilon membranes, the 40-kDa protein spot was identified by parallel immunoblotting with MAb 13C5 and excised from the blot. The N-terminal amino acid sequence of the protein was determined by automated Edman degradation on an Applied Biosystems 473A protein sequencer by S. Kielland, Department of Biochemistry and Microbiology, Victoria, British Columbia, Canada.

Cloning and sequencing of *pilT* genes. Thermal cycle dideoxy DNA sequencing was done according to the manufacturer's specifications (Circumvent; New England Biolabs) with custom-designed primers. DNA sequencing of plasmid clones was performed by the dideoxy chain-termination method using a modified form of T7 polymerase (29). 7-Deaza-dGTP (Pharmacia) was used in the place of dGTP to reduce the number of sequencing artifacts. The *pilT* gene from strain 2170 was cloned after PCR amplification using primers 1 (5′-GTTGA AACCCCTCCAGTC-3′) and 2 (5′-GTTTGCGCCTTGTTTT C-3′). *Taq* polymerase (Perkin-Elmer/Cetus) was used according to the manufacturer's specifications. Final PCR products were purified by agarose gel electrophoresis, end filled, and ligated into the compatible *Sma*I site of plasmid pBluescript KS+. Various DNA fragments were subcloned into pBluescript KS+ at convenient endonuclease sites. The λgt11 bank derived from *N. gonorrhoeae* R10 genomic DNA was kindly provided by Emil Gotschlich, and immunological screening was performed by the method described by his group (12). DNA and protein sequence data were compiled and analyzed with a computer using both the MacVector 3.5 (International Biotechnologies Inc.) and University of Wisconsin Genetics Computer Group (UWGCG) software packages (8). DNA homologies were found with the FASTA routine, and protein homologies were identified with TFASTA. Pairwise alignments of proteins were performed with the GAP program and default parameters.

Construction of gonococcal PilT mutants. Defined lesions in the *pilT* gene were constructed by utilizing a single *Eco*RI cleavage site present in plasmid p2A2 DNA (18) within the codons for amino acid residues 164 and 165 (see Fig. 4). The $pilT_{fs164}$ allele was created by filling in the 3′ recessed ends generated by *Eco*RI cleavage with the Klenow fragment of DNA polymerase I according to the manufacturer's specifications (New England Biolabs) followed by intramolecular ligation. The *pilT*::*erm* allele was created by insertion of an erythromycin resistance gene cassette (26) at the *Eco*RI site. Both mutations result in the expression of a truncated PilT polypeptide lacking residues. Details of the isolation and characterization of *N. gonorrhoeae* VD300 transformants expressing these *pilT* alleles will be described elsewhere.

Nucleotide sequence accession numbers. The sequence shown in Fig. 4 has been submitted to the GenBank data base under accession number L11719.

RESULTS

Isolation and characterization of MAb 13C5. A series of mouse hybridomas obtained following immunization of mice with whole gonococcal cells of strain 2170 were screened by whole-cell ELISA. By Western blotting, most of the selected MAbs reacted with previously identified surface antigens of *N. gonorrhoeae*. These included MAbs with reactivities to lipooligosaccharide, the porin protein (PI), and the lipoprotein H8. One MAb of the IgG1 isotype, designated 13C5, reacted with a gonococcal protein with a molecular size of 40 kDa. This antigen was present in all 165 strains of *N. gonorrhoeae* tested by whole-cell ELISA, and in all instances, a protein of 40 kDa was detected by Western blotting (Fig. 1). The protein bearing the epitope recognized by MAb 13C5 was also expressed at a comparable level among the diverse gonococcal isolates. The distribution of the epitope-bearing antigen among other bacterial species was examined by Western blotting. Only *N. meningitidis* (9 of 10), *Neisseria sicca*, and *Neisseria lactamica* were positive, with a 40-kDa protein species being detected in each case (Table 1).

Subcellular localization of the 40-kDa protein in *N. gonorrhoeae*. To determine the subcellular location of the 40-kDa protein in *N. gonorrhoeae*, cells from strain 2170 were harvested, disrupted, and fractionated into total membranes and

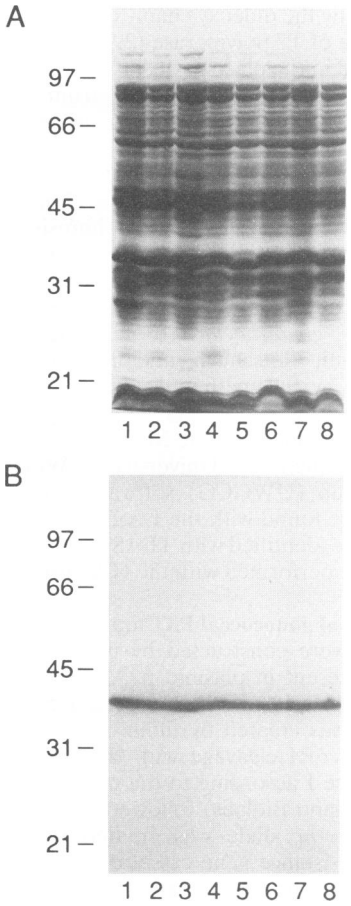

FIG. 1. Immunoblot analysis of proteins from eight different strains of *N. gonorrhoeae* with MAb 13C5. (A) Coomassie blue-stained gel; (B) immunoblot with anti-40-kDa MAb 13C5. Lanes: 1, strain 2140; 2, strain 2871; 3, strain 3232; 4, strain 2170; 5, strain 2609; 6, strain 2304; 7, strain 2719; 8, strain 2865. Molecular sizes (in kilodaltons) are indicated on the left.

soluble cytosolic proteins. Total-membrane fractions were treated with Sarkosyl and separated into soluble (cytoplasmic membrane) and insoluble (outer membrane) components. The proteins in these preparations were separated by SDS-PAGE and subsequently analyzed by immunoblotting with MAb 13C5. The 40-kDa protein was detected in both cytosolic and total-membrane fractions (Fig. 2B, lanes 2 and 3) and appeared to be present in the putative cytoplasmic membrane but not in the outer membrane fraction (Fig. 2B, lanes 4 and 5).

N-terminal sequencing of the 40-kDa protein. N-terminal amino acid sequencing was also used as a step toward directly characterizing the 40-kDa protein. Purified cytoplasmic membrane proteins derived from strain 2170 were separated by two-dimensional SDS-PAGE, with the protein being localized precisely by immunoblotting with MAb 13C5. It was readily apparent from the Coomassie-stained gels that the 40-kDa protein was a minor component of the polypeptide species present in the cytoplasmic membrane (Fig. 3). Although the identity of the first residue could not be ascertained, residues 2 to 17 were unambiguously determined by sequential Edman degradation of the protein transferred to a polyvinylidene difluoride membrane. A data base search with this sequence using the TFASTA program revealed only one significant match, that being the amino-terminal residues of the *P.*

TABLE 1. Reactivity of MAb 13C5 with different bacteria

Species[a]	No. reacting positive/no. tested by Western blotting[b]
N. meningitidis	9/10
N. lactamica	1/1
N. sicca	1/1
N. mucosa	0/2
N. cinerea	0/1
E. coli	0/1
P. aeruginosa	0/1

[a] The following bacterial strains were used in this study: *N. meningitidis*, ATCC 13090 and ATCC 13077 from the American Type Culture Collection and 188, 189, 193, 194, 196, 197, 198 and 199 from the Laboratory of Microbiology of the Centre Hospitalier de l'Université Laval, Quebec, Canada; *N. lactamica*, ATCC 23970; *N. sicca*, ATCC 29259; *N. mucosa*, ATCC 19696 and ATCC 25999; *N. cinerea*, ATCC 14685; *E. coli*, ATCC 25922; *P. aeruginosa*, ATCC 27853.

[b] Positive reactivity refers to the presence of a band for the strain tested with an apparent molecular size of 40 kDa on a Western blot probed with MAb 13C5 and ^{125}I-labeled anti-mouse immunoglobulin.

aeruginosa pilT gene open reading frame (ORF), with 11 of 16 residues being identical (Fig. 4).

Identification of the 40-kDa protein as gonococcal PilT. In order to identify directly the gene encoding the 40-kDa

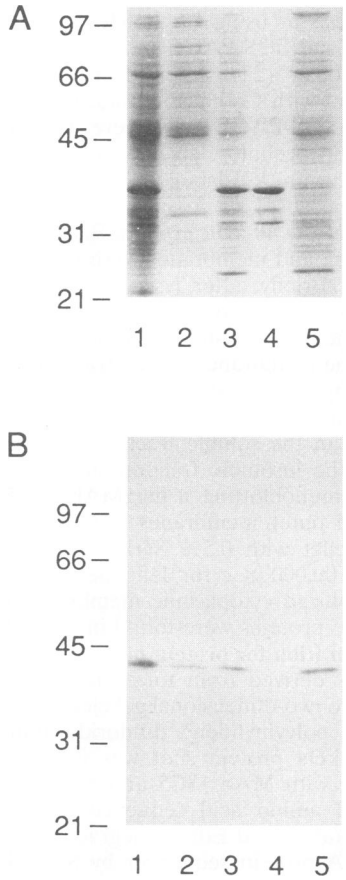

FIG. 2. Localization of the 40-kDa protein in *N. gonorrhoeae*. (A) Coomassie blue-stained gel; (B) autoradiogram of immunoblot with MAb 13C5. Lanes: 1, whole cells; 2, cytosolic fraction; 3, total membranes; 4, outer membrane (non-Sarkosyl-soluble fraction); 5, cytoplasmic membrane (Sarkosyl-soluble fraction). Molecular sizes (in kilodaltons) are indicated on the left.

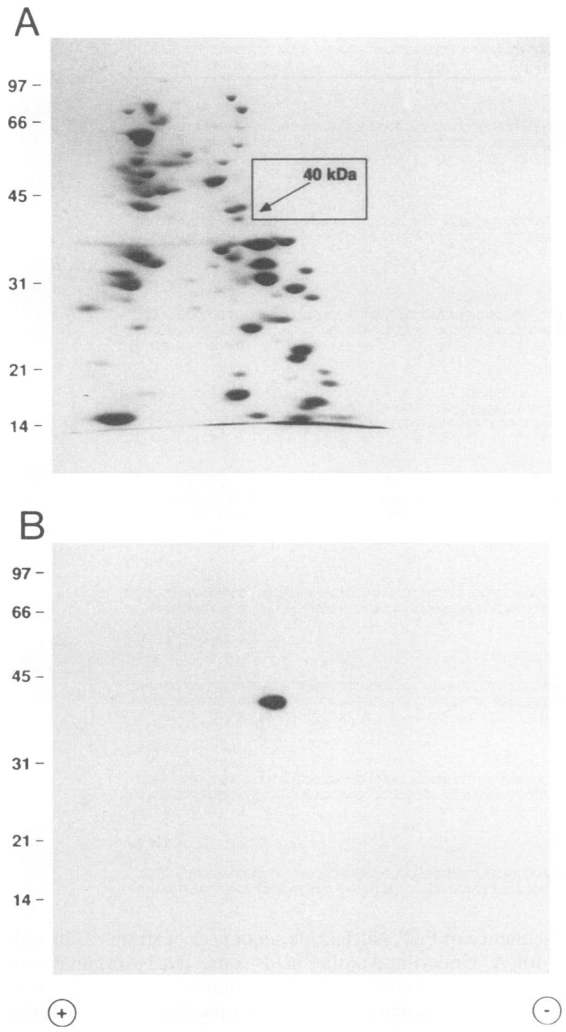

FIG. 3. (A) Two-dimensional SDS-PAGE of cytoplasmic membrane proteins from *N. gonorrhoeae*. Proteins from strain 2170 were electrophoresed in the first dimension with ampholytes in the pH range of 3 to 10 and in the second dimension by SDS-PAGE with 12% polyacrylamide. After electrophoresis, the gel was fixed and stained with Coomassie blue. (B) autoradiogram of immunoblot developed with MAb 13C5. Molecular sizes (in kilodaltons) are indicated on the left.

protein, plaques demonstrating immunoreactivity with MAb 13C5 were isolated from a λgt11 gene expression library created with genomic DNA from *N. gonorrhoeae* R10 (obtained from E. C. Gotschlich). Two such clones were plaque purified, their phage DNAs were prepared, and the gonococcal DNA inserts (*Eco*RI fragments of 800 and 600 bp, respectively) were subcloned into the pBluescript plasmid vector. Nucleotide sequencing of these subclones revealed that they contained identical ORFs capable of encoding a 186-amino-acid polypeptide which would have been translationally fused to the β-galactosidase gene of λgt11. When the TFASTA program was used, the sequence of the polypeptide derived from the ORF was found to be absolutely identical to the carboxy-terminal 186 amino acids of the previously described ORF of the *pilT* gene of *N. gonorrhoeae* MS11. This evidence appeared to indicate that the 40-kDa protein recognized by MAb 13C5 was the PilT protein. However, its N-terminal sequence did not match that derived from the gonococcal *pilT* ORF but did show strong identity with that derived from the *P. aeruginosa pilT* ORF. Moreover, no similarity between the N-terminal protein sequence and other reading frames at the 5′ end of the gonococcal *pilT* gene was found, ruling out a minor sequencing error as an explanation.

The possibility that gene rearrangements or other cloning artifacts might have led to errors in the *pilT* gene sequence was addressed by comparing Southern hybridization patterns using a *pilT* gene probe and DNA from p2A2 (the plasmid clone from which the original gonococcal DNA sequence was determined), λ clone 18/4 (the phage clone from which p2A2 was constructed), and *N. gonorrhoeae* MS11 (18). While the patterns for the *pilT* loci in λ clone 18/4 and the genomic DNA were identical, the gene carried on plasmid p2A2 had undergone an obvious rearrangement encompassing its 5′ end (data not shown). Furthermore, PCR using oligonucleotide primers complementary to sequences flanking the gene in p2A2 yielded products when that plasmid was used as a template but not when the λ clone 18/4 DNA and genomic DNA were used. Since it appeared that this rearrangement had occurred concurrently with subcloning onto the high-copy-number plasmid vector, the *pilT* gene was directly sequenced by thermal cycle dideoxy sequencing using the phage DNA clone as a template. The DNA sequence resulted in a deduced PilT polypeptide whose amino terminal residues matched perfectly those obtained by microsequencing of the 40-kDa protein (Fig. 4). PCR and thermal cycle DNA sequencing using the MS11 genomic DNA as a template and primers complementary to the new gene sequence confirmed that the *pilT* genes present in the genomic DNA and the lambda phage clone were identical.

The complete *pilT* gene of strain 2170 was then isolated by PCR amplification, and its nucleotide sequence was determined. The sequence was identical to that derived from strain MS11 save for a single-base change at position 661 (Fig. 4), which does not result in an amino acid change, and this nucleotide alteration was also present in the sequence obtained from the R10-derived λgt11 clones. The primary structures of the PilT proteins of strains MS11 and 2170 and the partial sequence for the strain R10 protein were thus absolutely conserved.

Gonococcal PilT mutants fail to react with MAb 13C5. Although these results indicated quite strongly that the 40-kDa protein reacting with the MAb 13C5 was indeed PilT, we sought to unequivocally confirm this by examining the reactivity of gonococcal mutants carrying defined mutations in the *pilT* gene. One gonococcal mutant had a frameshift mutation engineered into a unique *Eco*RI site, while the other was created by insertion of an erythromycin resistance-encoding gene cassette into that same restriction site (Fig. 4). These alterations result in the expression of a PilT protein lacking its 186 carboxy-terminal residues. Immunoblotting of the two gonococcal mutants and their respective isogenic parents revealed that 13C5 reactivity was abolished in the PilT mutants (Fig. 5).

DISCUSSION

Many investigators have sought to identify and characterize conserved surface components of *N. gonorrhoeae* in an effort to understand the immunobiology of gonococcal infection and to develop rational approaches for therapeutic interventions. In the course of analyzing MAbs elicited by immunization with whole gonococcal cells, the MAb 13C5 was found to react with all gonococcal strains tested and to recognize a protein antigen migrating with a molecular size of 40 kDa. Given the uniform

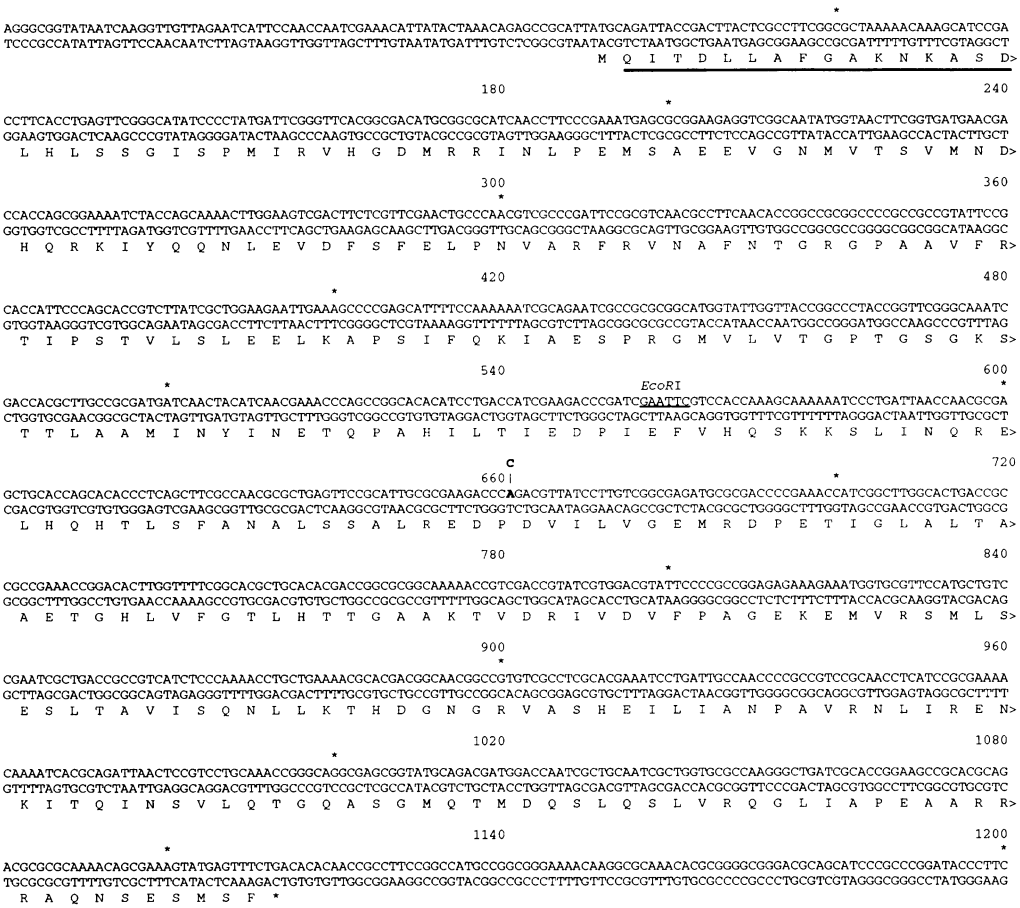

FIG. 4. Nucleotide sequence of the MS11 gonococcal *pilT* gene and amino acid sequence of PilT. Nucleotide sequences of strains 2170 and R10 are identical to that shown here except for nucleotide 661, where C is substituted for A. Underlined amino acids represent N-terminal residues identified by Edman degradation of the purified protein. The *Eco*RI restriction site used in construction of the PilT mutants and defining the fusion point found in the λgt11 clones is underlined at nucleotide positions 557 to 562. Nucleotide sequence 1 to 148 (encompassing amino acid residues 1 to 26) represents the gene segment corrected in these studies.

expression of the 13C5 epitope, the highly conserved character of the antigen, and the fact that a protein of this type had not previously been described, we sought to identify the reactive species. The results of studies described here indicate clearly that the antigen detected by the MAb 13C5 is in fact the gonococcal PilT protein.

Although the gonococcal *pilT* gene was originally found in a search to identify gonococcal genes sharing significant homology with the *pilB* gene of *P. aeruginosa*, its product is most similar to the potential product of the *P. aeruginosa pilT* gene (18). The latter gene was identified by virtue of its ability to phenotypically complement a *Pseudomonas* pilus mutant which retained piliation but was altered in the pilus-associated properties of twitching motility, colony morphology, and susceptibility to bacteriophage infection (3, 36). The basis for the association between these phenotypes, pili, and the putative product of the *pilT* gene is not known. The gonococcal PilT protein and the potential *P. aeruginosa* gene product both display canonical nucleotide binding sites, and according to the corrected sequence obtained in these studies, they share 66.6% amino acid identity and 81.4% similarity (Fig. 6).

Gonococcal PilT protein also shows significant identity with a distinct subset of proteins with conserved nucleotide binding domains and which are essential for transport of macromolecules across membranes (27). Members of this protein family lack a recognizable secretion signal sequence and stretches of hydrophobic residues that could act as membrane-spanning domains, suggesting that they are cytoplasmically localized. We observed, however, that the gonococcal PilT protein is found in both cytosolic and cytoplasmic membrane fractions. One other member of this family, the VirB11 protein of *Agrobacterium tumefaciens*, has been shown to be localized to both the cytoplasm and the cytoplasmic membrane fractions, with a barely detectable amount being present in the outer membrane preparations (6). In the latter studies, it was likewise demonstrated by N-terminal sequencing that the VirB11 protein (expressed in *E. coli*) was not proteolytically processed at the amino terminus. These sets of results are reminiscent of those found for the *E. coli* SecA protein, which associates with membranes in the absence of any segment of significant hydrophobicity (19) and contains the Walker box A motif of nucleoside triphosphate-binding proteins. They are also similar to the findings for less closely related members of the ATP-binding transport protein superfamily, which include proteins involved in the periplasmic binding protein-dependent transport of low-molecular-weight products (2). While caution must be taken in assessing evidence of protein localization due to possible methodological artifacts, our data lend credence to

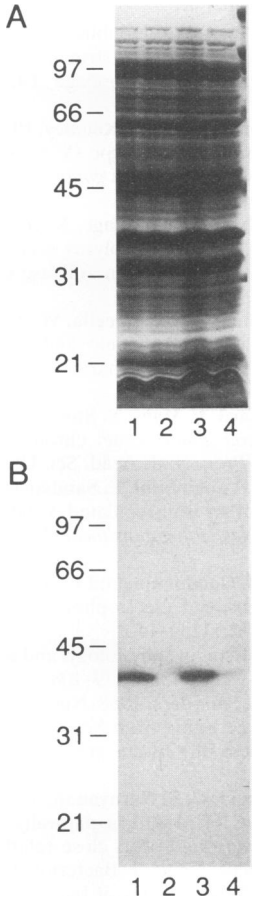

FIG. 5. Confirmation that the 40-kDa and PilT proteins are identical. (A) Coomassie blue-stained gel; (B) immunoblot with MAb 13C5. Lanes: 1, VD300; 2, VD300 *pilT*$_{fs164}$; 3, VD300; 4, VD300 *pilT*::*erm*. Molecular sizes (in kilodaltons) are indicated on the left.

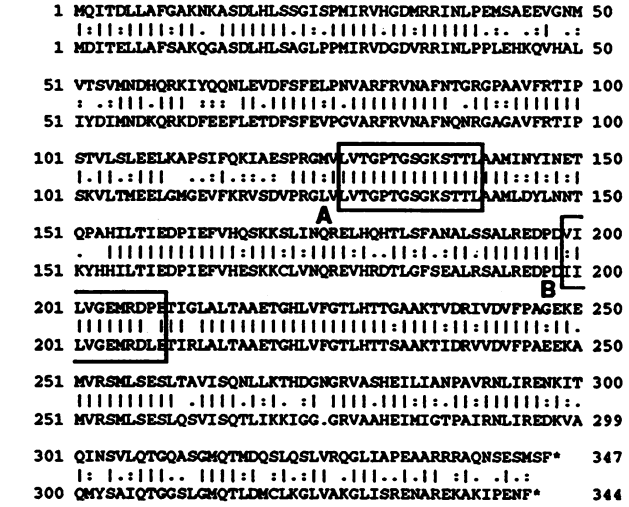

FIG. 6. Comparison of *N. gonorrhoeae* PilT and *P. aeruginosa* PilT. The GAP program of the UWGCG package was used to compare the deduced amino acid sequences of *N. gonorrhoeae* PilT (upper line) and *P. aeruginosa* PilT (lower line). Identical residues are indicated by vertical lines, and related residues are indicated by colons and periods. The boxed residues represent regions homologous to the type A and B domains proposed to be a part of a nucleotide binding site.

the notion that physical interaction between members of this protein family and the cytoplasmic membrane is essential to the functionality of their cognate systems.

The localization of gonococcal PilT to the intracellular milieu is somewhat at odds with the finding of immunoreactivity in whole-cell ELISA and the fact that the MAb 13C5 was selected after immunization with whole cells. However, the results could be explained most easily by the autolytic nature of the gonococcus (13). Additionally, the structural invariance and conserved gene sequence found for PilT are inconsistent with its being surface exposed. We are currently evaluating the precise subcellular deposition of gonococcal PilT by immunoelectron microscopy using the MAb 13C5.

A key finding stemming from this work is that the *pilT* gene clone characterized originally had undergone a gross rearrangement encompassing its 5′ end. This alteration arose during subcloning of the gene from a phage lambda clone to a high-copy-number plasmid vector. Some factors associated with this gene or its flanking sequence appear to preclude the maintenance of plasmids bearing this locus in *E. coli* since we have been unsuccessful in recovering clones carrying this segment of the gonococcal genome from a low-copy-number cosmid library. A large ORF whose derived polypeptide was structurally related to the carboxy-terminal portion of PilT (31% identity over 154 amino acid residues) was found upstream and in the opposite relative orientation in the rearranged clone, and a 15-bp inverted repeat sequence structure containing the gonococcal DNA uptake sequence was positioned immediately downstream of that ORF (18). The precise location of these *pilT*-related sequences which are present in the λ 18/4 clone and their potential contribution to the rearrangement and cloning difficulties are under investigation.

In summary, we have shown that the PilT protein of *N. gonorrhoeae* is expressed and conserved in its fundamental structure in all strains tested. It is worth noting that strain ATCC 13077 of *N. meningitidis* did not show reactivity with the MAb 13C5. Genomic DNA from this strain yielded the product of the predicted size with *pilT*-specific oligonucleotide primers. Nucleotide sequencing of the PCR products revealed a single-nucleotide difference from that found for *N. gonorrhoeae* 2170 in the region of the gene known to encode the MAb 13C5 epitope (amino acid residues 275 to 347 [16]). This creates a substitution of valine for alanine at residue 334, and it is tempting to speculate that this polymorphism accounts for the MAb 13C5 nonreactivity.

Although these studies indicate that all *N. gonorrhoeae* strains tested expressed PilT, the data concerning the distribution of PilT in other bacterial species are limited since only the distribution of the MAb 13C5 epitope was examined. Clearly, strong evidence suggests that *P. aeruginosa* expresses a highly related polypeptide (36) which does not appear to react with MAb 13C5. In fact, we would anticipate that many other species, particularly those which express type IV pili or related pili, will express homologs of PilT. However, the definitive detection of these putative homologs will require the use of precise gene probes and hybridization conditions or monospecific polyclonal antibodies since the *pilT* gene and gene product show significant nucleotide sequence and protein homology with related but clearly distinct members of the ATP binding cassette proteins (18, 27).

Our studies also revealed that the PilT protein can be recovered from both the cytoplasmic and the cytoplasmic

membrane fractions. The potential for localization to the cytoplasmic membrane was not readily apparent from the overall hydrophilic character of the protein but is consistent with a model suggesting a direct role for this protein in membrane-associated transport phenomena. Finally, the availability of the MAb 13C5 and the viability of gonococcal mutants which express grossly truncated PilT polypeptides will facilitate assessments of the role this protein may play in the expression of gonococcal pili and associated properties.

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