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Antibodies to peptides corresponding to a conserved sequence of gonococcal pilins block bacterial adhesion

(immunogenicity/synthetic peptides/reverse turns/gonococcal vaccine)

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ABSTRACT **Antisera generated against each of seven synthetic peptides corresponding to constant and variable sequences of the pilin from gonococcal strain MS11 were assayed for their ability to crossreact with intact pili from both homologous and heterologous strains. The peptides elicited roughly equal antipeptide responses but varied substantially in their ability to elicit antisera that crossreacted with intact pili. Of the antisera to peptides corresponding to regions of conserved sequence, antisera directed against residues 69–84 were the most efficient in binding pili from all strains tested in both solid-phase assays and immunoblots. Anti-69–84 also efficiently precipitated a tryptic fragment of pilin known to bind human endocervical cells. Sera against the two peptides (121–134 and 135–151) previously shown to contain strain-specific epitopes crossreacted with MS11 pili equally well, but differed in their ability to bind pili from heterologous strains. Anti-121–134 was strain-specific whereas anti-135–151 bound all pilin tested. Each of the sera was examined for its ability to inhibit bacterial adhesion to a human endometrial carcinoma cell line. Sera generated against residues 41–50 and 69–84 successfully inhibited a heterologous gonococcal strain from binding. These peptides could be important components of an effective vaccine for the prevention of gonorrhea.**

The antigenic variation of the surface molecules of pathogenic organisms is an important obstacle to the development of efficacious vaccines for a variety of diseases (1–6). In only a few systems are the molecular details of the sequence variation of the membrane proteins and the genetic mechanism for their variation partially understood (7, 8).

Efforts to develop a successful vaccine against gonorrhea have concentrated on blocking the organism's binding to eukaryotic cell surfaces. Gonococcal adhesion to mucus membranes is mediated in part by pili, filamentous polymers that extend out from the bacterial surface (9). These organelles are composed of a single repeating protein subunit, pilin. Pili prepared from different gonococcal strains are antigenically heterogeneous and attempts to use them as components of a vaccine have resulted only in protection against the homologous strain (10). In an earlier investigation, we identified the linear strain-specific and common epitopes of gonococcal pilin (11). By using synthetic peptides corresponding to different regions of the protein, we showed that antisera against pili from strain MS11 are predominantly strain specific and directed at two epitopes within a disulfide loop near the carboxyl terminus of the pilin molecule. We also determined that the sequence of this region varies greatly between different strains of gonococci. In contrast, the conserved, receptor-binding portion of the sequence (residues 31–92) contained only a weakly immunogenic epitope, common to all pili from the strains examined, between residues 48 and 60. However, when the central cyanogen bromide-generated fragment (CNBr II, residues 8–92) was used as an immunogen, a much stronger crossreactive response was generated which was directed at a region between residues 69 and 84. This was the first indication that peptides from pilin can elicit a different population of antibodies than that produced when the intact protein is used as an immunogen.

In this study we have used as immunogens the same peptides previously used as antigens to map the antigenic structure of gonococcal pilin (11). Antisera generated against the synthetic peptides were examined for their ability to crossreact with intact pili from homologous and heterologous strains by solid-phase binding assays and immunoblots. The sera also were used to immunoprecipitate an iodinated tryptic fragment of pilin previously shown to bind human endocervical cells (12). Finally, sera against residues 41–50 and 69–84 separately were shown to inhibit intact bacteria from binding human endometrial carcinoma cells.

MATERIALS AND METHODS

Gonococcal Strains and Growth Medium. The isolation and maintenance of gonococcal strains MS11 and R10 have been described (12). Strain 1896 was isolated in Seattle from a patient with a disseminated gonococcal infection, whereas R16, F62, and 2686 were provided by the Cornell University School of Medicine, New York and D. S. Kellogg, Jr. (Center for Disease Control, Atlanta) from patients with uncomplicated gonorrhea. The four colonial variants [piliated/opaque (op) or transparent (t) and nonpiliated/opaque or transparent] of each strain were independently propagated on solid typing medium by selective daily passage of single colonies (13).

Purification of Pili. Pili were purified from MS11 (t) and R10 (t) strains of *N. gonorrhoeae* as described (12).

Selection and Synthesis of Peptides. The peptides corresponding to regions of MS11 pilin in this study were the same as those used to determine the antigenic structure of the molecule (11). Peptides were synthesized by solid-phase techniques (14) with a Beckman model 990B peptide synthesizer using commercially available amino acid polystyrene resins and *tert*-butoxycarbonyl-protected amino acids (Peninsula Laboratories, Belmont, CA).

Conjugation of the Peptides to Carrier Proteins. The peptides were conjugated to bovine serum albumin (BSA) with succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (Pierce) as described (11). For immunization, they were conjugated to thyroglobulin with *m*-maleimidobenzoyl *N*-hydroxysuccinimide ester (Pierce). The conjugates used in this study each contained 15–25 peptides per molecule of BSA and 25–35 peptides per 100 kDa of thyroglobulin.

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Abbreviation: BSA, bovine serum albumin.

Immunization Protocol. Subscapular and intramuscular immunizations of two rabbits were performed with 500 μg of each peptide-thyroglobulin conjugate in complete Freund's adjuvant. After 8 weeks, the animals were injected with the immunogen in incomplete Freund's adjuvant and bled 1 week later.

Solid-Phase Binding Assays. A solid-phase binding assay was used to determine the ability of the sera generated against each of the seven peptides to recognize MS11 pili, R10 pili, and the homologous peptide conjugated to BSA. Briefly, 96-well plates were coated with either peptide-BSA conjugate or intact pili at 100 μg/ml, washed, incubated with serially diluted antisera, washed, and incubated with ^{125}I-labeled protein A (Amersham). The wells were washed and cut from the plate, and bound radioactivity was measured.

Immunoblots. Intact colonies of gonococci grown on agar were removed with filter paper and lysed with $NaDodSO_4$/PAGE sample buffer. The lysates were loaded directly onto a 12% polyacrylamide/$NaDodSO_4$ gel and electrophoresed. The resolved bacterial proteins were electrophoretically transferred onto nitrocellulose (BA83, Schleicher and Schuell) and the membrane was treated with 95% P_i/NaCl (0.15 M NaCl/0.01 M sodium phosphate, pH 7.4)/5% skim milk at 37°C for several hours to saturate the matrix. The nitrocellulose was then incubated with appropriate dilutions of antipeptide sera, washed, exposed to 100,000 cpm of ^{125}I-labeled protein A, washed, dried, and autoradiographed.

Immunoprecipitations. TC-2 (residues 31–111), a receptor-binding tryptic peptide of pili, was generated, purified, and iodinated as described (12). Labeled TC-2 (30,000 cpm) in P_i/NaCl was incubated with various dilutions of each antipeptide sera and then combined with 100 μl of protein A-Sepharose (Pharmacia). The bound immunocomplexes were collected by centrifugation, washed, and assayed for ^{125}I.

Blocking Bacterial Adherence to Endometrial Cells. The human endometrial carcinoma cell line ENCA-4 was cultured originally from a patient with grade III adenocarcinoma of the endometrium. The cells were grown on coverslips in Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum. Piliated, transparent colonies of gonococcal strain F62 were harvested from agar plates, suspended to an OD_{660} = 0.14 in 50 mM Tris/50 mM sodium acetate/140 mM NaCl/5 mM $CaCl_2$/4 mM KCl/2 mM $MgCl_2$/0.1% BSA, pH 6.5. Dilutions of preimmune or antipeptide sera and bacteria were incubated at 37°C for 2 hr and added to the washed coverslips. The mixture was incubated with gentle rocking for 1 hr at 37°C, after which the coverslips were washed with P_i/NaCl, fixed with methanol, and treated with Giemsa stain. The number of bacteria bound per cell was determined visually.

Table 1. Amino acid sequences of the seven synthetic oligopeptides and their corresponding location in MS11 pilin

L	P	A	Y	Q	D	Y	T	A	R	A	Q	V	S	E	G	C			
21														35					
E	G	Q	K	S	A	V	T	E	Y	G	C								
41									50										
T	E	Y	Y	L	N	H	G	K	W	P	E	N	G	C					
48												60							
P	P	S	D	I	K	G	K	Y	V	K	E	V	E	V	K	G	C		
69															84				
S	L	W	A	R	R	E	N	G	S	V	K	W	F	C					
107														121					
C	G	Q	P	V	T	R	T	D	D	D	T	V	A						
121													134						
D	A	K	D	G	K	E	I	D	T	K	H	L	P	S	T	C			
135																151			

Corresponding sequence locations in MS11 pilin are given beneath the sequence of each peptide. Underlined residues do not exist in the primary structure of MS11 pilin; these glycine and cysteine residues were added as spacers and for attachment to carrier molecules, respectively. The single-letter amino acid code is used (15).

RESULTS

The MS11 synthetic peptide analogues used in this study (Table 1) correspond to regions both of conserved sequence (residues 21–35, 41–50, 48–60, and 69–84) and of variable sequence (residues 107–121, 121–134, and 135–151) between pilin from various gonococcal strains (12). Fourteen rabbits were immunized with the seven peptides conjugated to thyroglobulin (two rabbits per conjugate). The resultant antisera were screened for their ability to bind the homologous peptide conjugated to BSA as well as for their ability to crossreact with intact MS11 and R10 pili (Fig. 1). Similar results were obtained with the sera from both rabbits immunized with each peptide. Though not identical in their immunogenicity, all of the peptides elicit good antipeptide responses. However, they substantially differed in their ability to generate antibodies that crossreacted with the intact pilus proteins. Not surprisingly, the sera engendered by immunizing

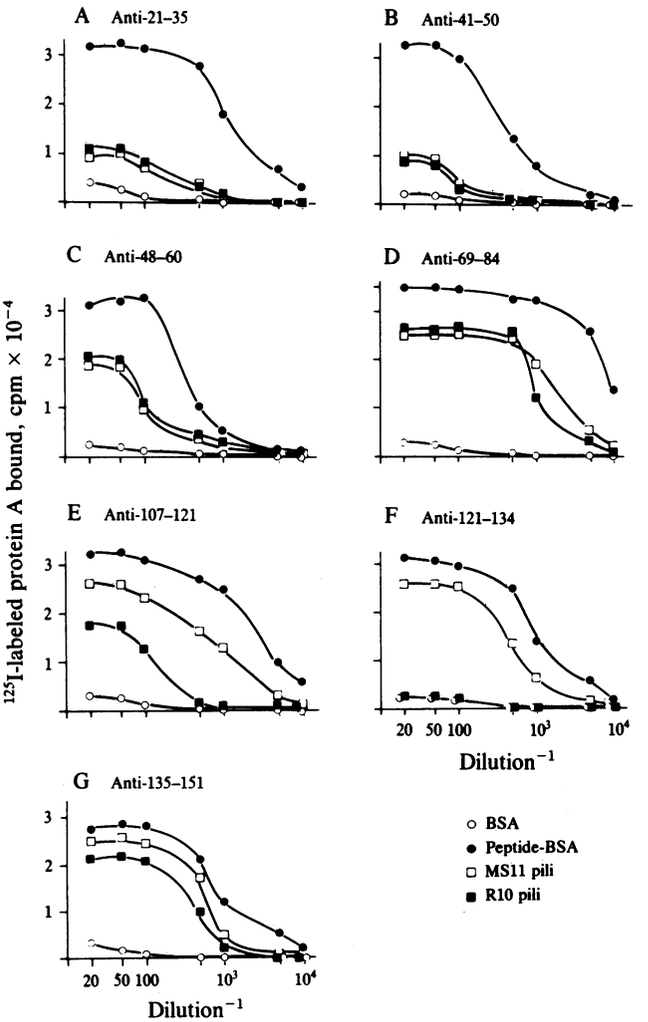

FIG. 1. The crossreaction of antipeptide sera with either homologous (MS11) or heterologous (R10) pili in solid-phase binding assays. Antisera generated to peptide-thyroglobulin conjugates corresponding to residues 21–35 (*A*), 41–50 (*B*), 48–60 (*C*), 69–84 (*D*), 107–121 (*E*), 121–134 (*F*), and 135–151 (*G*) of MS11 pilin were allowed to react with homologous peptide-BSA conjugate (●), intact MS11 pili (□), intact R10 pili (■), or BSA (○).

with peptides from regions of sequence identity between MS11 and R10 pili crossreacted equally well with the two proteins (Fig. 1 *A–D*). However, residues 69–84 are far more effective in evoking crossreactive antibodies than 21–35, 41–50, and even 48–60, a natural epitope of gonococcal pilin (11). The three peptides from variable portions of the molecule, 107–121, 121–134, and 135–151, also are roughly equal in their ability to elicit crossreactive antibodies to MS11 pili but differ in their ability to bind R10 pili. Antibodies to 135–151 bound R10 almost as well as they did MS11 (Fig. 1*G*), whereas antisera to 121–134 were specific for MS11 (Fig. 1*F*). Antibodies against 107–121 were intermediate in their specificity, binding R10 only at low dilutions of antisera (Fig. 1*E*). Peptides 121–134 and 135–151 each contain a strain-specific epitope (11), yet when used as immunogens, 121–134 elicits a strain-specific response whereas 135–151 evokes antibodies that crossreact with heterologous pili.

The crossreactivity of the seven antipeptide antisera with intact pilin from MS11, R10, and three other gonococcal strains was examined by use of immunoblots. All of the sera bound MS11 pilin but to different extents. The strongest signals were seen with antisera to 69–84, 121–134, and 135–151 (Figs. 2–4). Antisera against 69–84 reacted with pilin from both opaque and transparent colonies of strains MS11, R10, F62, 1896, and 2686 (Fig. 2), as did sera from rabbits immunized with 135–151 (Fig. 3). In contrast, antibodies against 121–134 bound MS11 but not R10 pilin (Fig. 4); these sera crossreact only with heterologous pilin from strain F62 (data not shown).

To determine whether the sera against the peptides corresponding to the region of conserved sequence crossreact well enough with pili to be used as reagents to examine the biological functions of the protein, we examined their ability to immunoprecipitate TC-2 (residues 31–111). This peptide is prepared by tryptic digestion of citraconylated pilin and was shown previously to encompass a receptor-binding domain of gonococcal pili (12). Antisera to residues 21–35, 41–50, 48–60, and 69–84 all immunoprecipitate this fragment (Fig. 5). Sera directed against 69–84 precipitate TC-2 much more effectively than the other antisera, even at dilutions of 1:50,000.

Antisera to each peptide were screened for their ability to block the binding of viable gonococci derived from piliated, transparent colonial variants to human endometrial carcinoma cells grown as monolayers on cover slips. Endometrial cells were used because (*i*) gonococcal endometritis is a well described phenomenon (16); (*ii*) they more closely resemble the epithelial cell in natural infection than do erythrocytes, which had been used in a previous study (17); and (*iii*) they are more readily bound by piliated gonococci than are either HeLa or buccal cells. Strain F62 was employed in these assays because (*a*) of the heterologous strains examined, it displayed the greatest capacity to bind the ENCA-4 endometrial cells and consequently provided the most stringent test of the antisera's capacity to block attachment; (*b*) the antisera to both the conserved sequence of the molecule and the two peptides composing the disulfide loop crossreacted with F62 pilin on immunoblots, therefore allowing comparison of the effectiveness of antibodies against the conserved sequences with that of antibodies against the variable domain to inhibit binding; and (*c*) this strain has been shown to cause urethritis in male volunteers (18) and thus has retained its pathogenicity after *in vitro* passage. Only antisera to peptides 41–50 and 69–84 efficiently prevented bacterial attachment (Table 2). A 1:10 dilution of either serum was most effective, but a 1:50 dilution of anti-41–50 and a 1:100 dilution of anti-69–84 inhibited ≈90% of the binding. In contrast, antisera to the other two peptides of the constant region (residues 21–35 and 48–60) did not block attachment. Interestingly, antisera against the two immunodominant regions of intact pili (residues 121–134 and 135–151), previously shown to crossreact with F62 pilin on immunoblots, did not inhibit the bacteria from binding the endometrial cells.

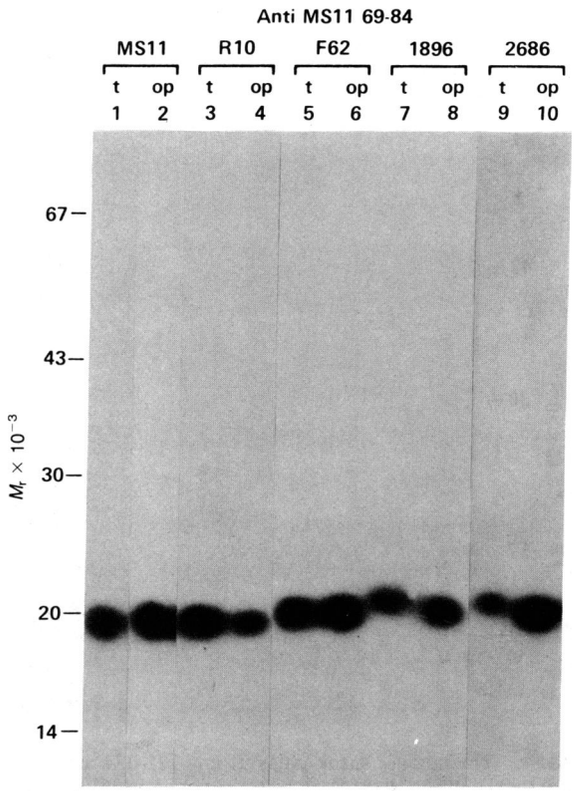

FIG. 2. Autoradiograph of an immunoblot displaying the binding specificity of antiserum to residues 69–84 for homologous and heterologous pilin. $NaDodSO_4$/PAGE-resolved samples of intact transparent (t) or opaque (op) gonococcal colonial variants from strains MS11 (lanes 1 and 2), R10 (lanes 3 and 4), F62 (lanes 5 and 6), 1896 (lanes 7 and 8), and 2686 (lanes 9 and 10) were probed with a 1:400 dilution of anti-69–84-thyroglobulin conjugate as described in *Materials and Methods*. In all cases the antibody specifically bound pilin. Positions of molecular weight standards are shown on the left.

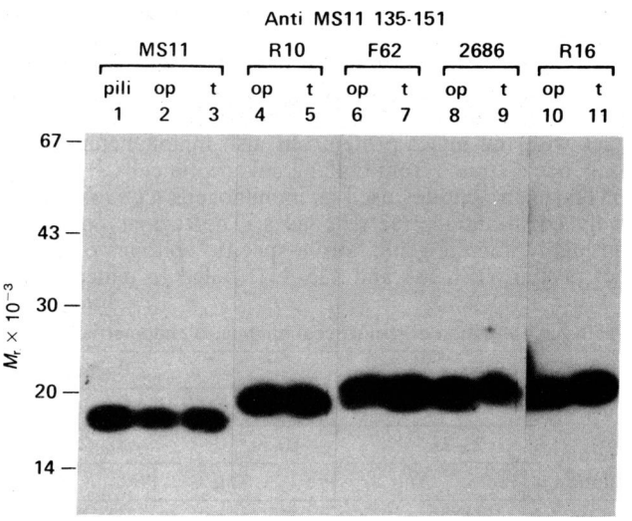

FIG. 3. Autoradiograph of an immunoblot displaying the binding specificity of anti-135–151 for homologous and heterologous pilin. A 1:400 dilution of antisera was used to probe transparent (t) or opaque (op) variants of piliated colonies of strain MS11 (lanes 2 and 3), R10 (lanes 4 and 5), F62 (lanes 6 and 7), 2686 (lanes 8 and 9), and R16 (lanes 10 and 11). Purified MS11 pili were electrophoresed in lane 1. In all cases the sera specifically bound pilin.

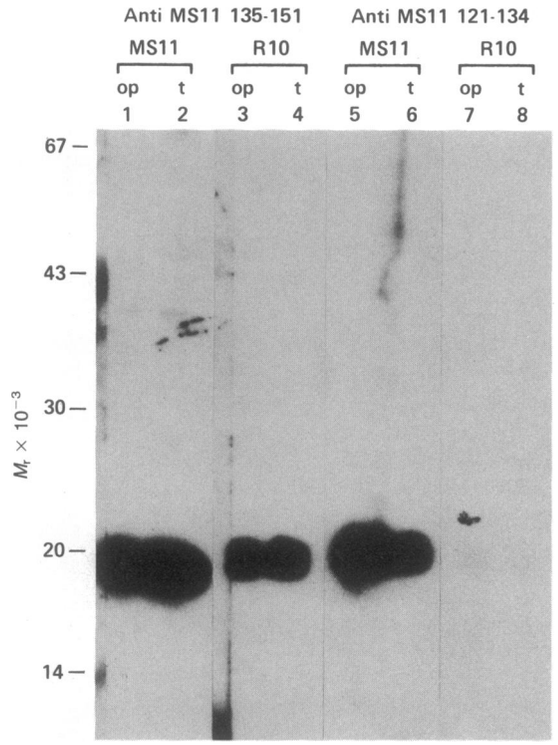

FIG. 4. The binding specificities of anti-121–134 and anti-135–151. A 1:400 dilution of either anti-121–134 or anti-135–151 was used to probe immunoblots of transparent (t) or opaque (op) piliated colonies of strain MS11 (lanes 1, 2, 5, and 6) and R10 (lanes 3, 4, 7, and 8).

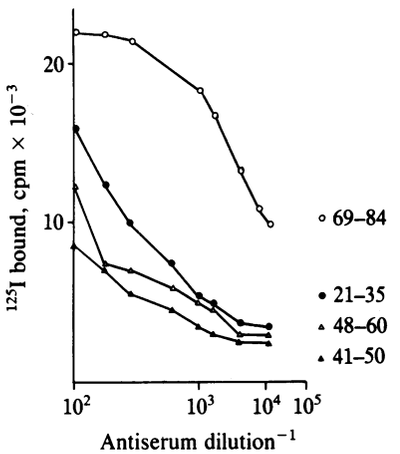

FIG. 5. Immunoprecipitation of ^{125}I-labeled peptide TC-2 (residues 31–111) with antipeptide sera. Iodinated TC-2 (50,000 cpm) was incubated with various dilutions of anti-21–35 (●), anti-41–50 (▲), anti-48–60 (△), or anti-69–84 (○) as described in *Materials and Methods*.

DISCUSSION

A pathogen's ability to evade the host immune response by antigenic variation provides an intriguing system for study of the molecular details of the antigenicity and immunogenicity of proteins. When rabbits are immunized with purified gonococcal pili, the majority of antibodies are directed at two strain-specific epitopes within a disulfide loop between residues 121 and 151 (11). Consequently, pili prepared from a single strain probably cannot be used as a broadly protective gonorrhea vaccine.

Experiments described in this paper provide evidence that synthetic peptides corresponding to weakly immunogenic regions of the protein can elicit antibodies that not only crossreact with the intact protein but also inhibit heterologous gonococcal strains from binding eukaryotic cells.

The seven peptides used as immunogens all evoked relatively equal antipeptide responses. The regions containing the highly immunogenic, strain-specific epitopes of the intact protein (121–134 and 135–151) failed to generate any greater antipeptide response than the other five peptides. However, antisera elicited by each of the seven peptide analogues of gonococcal pilin substantially differ in their ability to crossreact with the intact protein when assayed either by solid-phase binding, immunoblotting, or immunoprecipitation. The variation in the peptides' ability to generate a crossreactive response is best explained by the number and type of B-cell clones the peptides stimulate. When a synthetic peptide is used as an immunogen, the possible conformations that it can adopt will determine which clones in the B-cell repertoire are stimulated to produce antibodies. Only if the peptide adopts a secondary structure identical to that it assumes in the intact protein will it elicit antibodies that crossreact with that protein. We believe that the peptides that generate the highest anti-pili response (69–84, 121–134, and 135–151) have the highest probability of existing in the identical conformation they assume in pilin or have the lowest energy requirements for being induced to adopt the correct conformation by a complementary antibody combining site.

The regions of pilin that were synthesized were chosen based on their likelihood to adopt structures similar to their predicted conformation in the intact protein. Each peptide has a predicted reverse turn located distal to its site of linkage to the carrier protein. We believe that of the three known secondary structures—α-helix, β-sheet, and reverse or β-turns—reverse turns have the highest probability of occurring in a peptide <20 amino acids long. This conclusion is based on the supposition that the forces involved in sheet and helix formation are primarily found in the tertiary structure of the protein. Such interactions as inter-strand hydro-

Table 2. Inhibition of gonococcal binding to endometrial cells by synthetic peptide antisera

Serum dilution	Bacteria per cell, mean ± standard deviation													
	Constant region immunogens								Variable region immunogens					
	21–35		41–50		48–60		69–84		107–121		121–134		135–151	
	Pre	Anti	Pre	Anti	Pre	Anti	Pre	Anti	Pre	Anti	Pre	Anti	Pre	Anti
1:10	47 ± 14	12 ± 10	32 ± 8	3 ± 1	48 ± 14	56 ± 15	26 ± 9	1 ± 1	42 ± 13	48 ± 19	61 ± 16	72 ± 10	80 ± 13	73 ± 22
1:25	41 ± 7	56 ± 9	65 ± 15	9 ± 4	ND		48 ± 10	8 ± 3	60 ± 12	72 ± 11	63 ± 14	58 ± 9	74 ± 18	99 ± 23
1:50	ND		83 ± 9	9 ± 5	ND		170 ± 21	10 ± 4	ND		49 ± 11	58 ± 18	76 ± 12	52 ± 11
1:100	ND		54 ± 10	49 ± 12	ND		94 ± 11	11 ± 2	ND		ND		89 ± 11	89 ± 26
1:200	ND		38 ± 8	45 ± 8	ND		41 ± 9	46 ± 7	ND		ND		ND	

Binding was determined for gonococci preincubated with preimmune serum (Pre) or with antiserum (Anti) to synthetic peptides corresponding to the indicated sequences of MS11 pilin. ND, not done.

gen bonding in sheets and hydrophobic bonds found in helix-packing and sheet–sheet interactions will be absent in a linear peptide. The structural forces responsible for reverse turns are much simpler. Turns invariably are composed of hydrophilic residues prominantly exposed on the surface of globular proteins, often with the first and fourth amino acids associated with a hydrogen bond (19). Usually they are flanked by hydrophobic residues whose association is thought to be the driving force for turn formation (20). Consequently, relatively short linear peptides can contain all the residues involved in turn formation.

Based on the mechanism of adhesion of other gram-negative bacteria (21–23), pilin is most likely a lectin that binds a carbohydrate moiety on the eukaryotic cell surface. A high percentage of the residues that hydrogen-bond the ligand in the binding sites of the few carbohydrate-binding proteins whose three-dimensional structures have been determined (4, 24–27) are present in turns. We felt that an antibody generated against a predicted turn in the conserved region of gonococcal pilin might block pili from binding eukaryotic membranes. These ideas appear to be correct because antibodies elicited by residues 41–50 and 69–84 from the constant portion of MS11 pilin inhibit strain F62 from attaching to endometrial cells *in vitro*. In contrast, attachment is not blocked by antibodies to two peptides distal to the receptor-binding segment: 121–134, which shares an epitope with F62 (data not shown), and 135–151, which elicits high-titer, crossreacting antibodies. Thus we propose that regions 41–50 and 69–84 are involved in receptor binding. The inhibition data also support our previous prediction that antibodies directed against the highly immunogenic regions within the disulfide loop would not block adhesion (11).

Additional evidence that the immune response against peptides is distinct from that against the intact protein is the specificity of the antibodies generated against residues 135–151. In the intact protein this region elicits antibodies that are specific for MS11 pili. In contrast, the isolated peptide generates antibodies that bind not only MS11 pili but also pili from all other strains tested. Comparison of the sequences of pilins from different gonococcal strains indicates that residues 134–145 are variable but residues 146–151 are constant. That two separate epitopes can exist within a peptide this size is not surprising but rather that only the variable region is immunogenic when the intact protein is used as an immunogen, whereas both the variable and conserved regions are immunogenic when the peptide is used. We assume that the different responses are due to events involved in antigen presentation. Free antigen does not stimulate B-cells directly. Only when antigen is associated with immune-response gene products and the T-cell receptor is it capable of stimulating a humoral response (28). These components of the immune system must associate with pilin and may prevent portions of its surface from being seen by the B-cell population.

The results presented in this report are consistent with earlier observations by Tramont *et al.* (29) that an intact pilus vaccine elicits receptor-blocking antibodies directed at a common epitope, since they could be absorbed with heterologous pili. In contrast, Virji and Heckels (30) have recently reported that strain-specific but not crossreacting monoclonal antibodies block attachment, although the location of their epitopes was not determined. Taken as a group, these studies and the data presented here suggest that both linear and nonlinear strain-specific and common epitopes probably exist and that representatives of each may elicit receptor-blocking antibodies depending on their spatial proximity to the residues involved in ligand binding in the folded pilin molecule. These considerations notwithstanding, by eliciting sequence-specific, functionally defined antibodies, the synthetic peptides described in this report have enabled us to extend our previous structure–function analysis of pili. The peptides that elicit crossreacting, receptor-blocking antibodies are promising candidate immunogens for the prevention of gonorrhea.

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1. Cross, G. A. M. (1978) *Proc. R. Soc. London, Ser. B* **202,** 55–72.
2. Gething, M., Bye, J., Skehel, J. & Waterfield, M. (1980) *Nature (London)* **287,** 306–310.
3. Krystal, M., Young, J. F., Palese, P., Wilson, I. A., Skehel, J. J. & Wiley, D. C. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4527–4531.
4. Colman, P. M., Varghese, J. N. & Laver, W. G. (1983) *Nature (London)* **303,** 41–44.
5. Dale, J., Ofek, I. & Beachey, E. H. (1980) *J. Exp. Med.* **151,** 1026–1038.
6. Barbour, A. G., Tessier, S. L. & Stoenner, H. G. (1982) *J. Exp. Med.* **156,** 1312–1324.
7. Borst, P. & Cross, G. A. M. (1982) *Cell* **29,** 291–303.
8. Webster, A. G., Laver, W. G., Air, G. M. & Schild, G. C. (1982) *Nature (London)* **296,** 115–121.
9. Swanson, J. (1973) *J. Exp. Med.* **137,** 571–589.
10. Brinton, C. C., Jr., Bryan, J., Dillon, J. A., Guerina, N., Jacobson, L. J., Kraus, S., Labik, A., Lee, S., Levene, A., Lim, S., McMichael, J., Polen, S., Rodgers, K., To, A. C. & To, S. C. (1978) in *Immunobiology of Nisseria gonorrhoeae*, eds. Brooks, G. F., Holmes, K. K., Sawyer, W. D. & Young, F. E. (Am. Soc. Microbiol., Washington, DC), pp. 155–178.
11. Rothbard, J. B., Fernandez, R. & Schoolnik, G. K. (1984) *J. Exp. Med.* **160,** 208–221.
12. Schoolnik, G. K., Fernandez, R., Tai, J. Y., Rothbard, J. B. & Gotschlich, E. C. (1984) *J. Exp. Med.* **159,** 1351–1370.
13. Swanson, J. (1978) *Infect. Immun.* **19,** 320–331.
14. Erickson, B. W. & Merrifield, R. B. (1976) in *The Proteins*, ed. Neurath, H. (Academic, New York) Vol. 2, pp. 255–527.
15. IUPAC-IUB Commission on Biochemical Nomenclature (1968) *Eur. J. Biochem.* **5,** 151–153.
16. Novak, A. B. & Woodruff, J. D. (1979) in *Gynecologic and Obstetric Pathology* (Saunders, Philadelphia), p. 319.
17. Schoolnik, G. K., Tai, J. Y. & Gotschlich, E. C. (1983) *Prog. Allergy* **33,** 314–331.
18. Kellogg, D. S., Cohen, I. R., Norens, L. C., Schroeter, A. L. & Reising, G. (1968) *J. Bacteriol.* **96,** 596–605.
19. Richardson, J. S. (1982) *Adv. Protein Chem.* **34,** 167–339.
20. Rose, G. D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4643–4646.
21. Salit, I. E. & Gotschlich, E. C. (1977) *J. Exp. Med.* **146,** 1169–1181.
22. Ofek, I., Mirelman, D. & Sharon, N. (1977) *Nature (London)* **265,** 623–626.
23. Gaastra, W. & deGraaf, F. K. (1982) *Microbiol. Rev.* **46,** 129–161.
24. Phillips, D. C. (1967) *Proc. Natl. Acad. Sci. USA* **57,** 484–495.
25. Becker, J. W., Reeke, G. N., Jr., Cunningham, B. A. & Edelman, G. M. (1976) *Nature (London)* **259,** 406–409.
26. Hardman, K. D. & Ainsworth, C. F. (1976) *Biochemistry* **15,** 1120–1128.
27. Newcomer, M. E., Gilliland, G. L. & Quiocho, F. A. (1981) *J. Biol. Chem.* **256,** 13213–13217.
28. McDevitt, H. O. & Benacerraf, B. (1969) *Adv. Immunol.* **11,** 31–74.
29. Tramont, E. C., Sadoff, J. C., Boslego, J. W., Ciak, J., McChesney, D., Brinton, C. C., Wood, S. & Takafuji, E. (1981) *J. Clin. Invest.* **68,** 881–888.
30. Virji, M. & Heckels, J. E. (1984) *J. Gen. Microbiol.* **130,** 1089–1095.