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Genital Antibody Response to a Parenteral Gonococcal Pilus Vaccine

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A parenteral gonococcal pilus vaccine which has previously been shown to be safe and antigenic also results in the production of specific local genital antibody. All three major antibody classes were present in the local secretions, but immunoglobulin A predominated, a portion of which is dimeric 11S immunoglobulin A. This mucosal antibody is also capable of blocking the attachment of gonococci to epithelial cells. The antibody cross-reacted with five heterologous pili in a solid-phase radioimmunoassay. These results are encouraging and suggest that a gonococcal pilus vaccine may be efficacious in preventing gonorrhea.

Infections with *Neisseria gonorrhoeae* are usually localized to mucosal surfaces. A local immune response to gonococcal infections has been demonstrated (10, 18). At least one of the functions of this locally produced antibody is to inhibit the attachment of gonococci to epithelial cells (14, 19).

Freshly isolated organisms and those most likely to produce an experimental infection in humans are piliated. Gonococcal pili isolated from organisms grown on artificial media are filamentous protein structures extending from the cell wall, and they have been shown to be the principal mediators of attachment to mucosal cells (6). Accordingly, a prototype gonococcal pilus vaccine has been proposed (3), manufactured (C. C. Brinton and J. C. McMichael, Chem. Abstr. 701332 **86**:427, 1976), and tested for safety and antigenicity (20). It has been postulated that if this vaccine is to be effective, antibodies must be present on the local genital mucosal surface (17, 19). The report herein describes the local genital antibody response induced by a parenteral gonococcal pilus vaccine administered to human volunteers.

MATERIALS AND METHODS

Gonococcal pilus vaccine. The gonococcal pilus vaccine was prepared at the University of Pittsburgh by C.C.B. (3a; Brinton and McMichael, Chem. Abstr. 701332 **86**:427, 1976).

Cloned, piliated-phase, transparent-colony type 2 organisms were harvested into 0.01 M phosphate-buffered saline (PBS) (pH 6.8) from the surface of solid GC medium (Difco Laboratories, Detroit, Mich.) enriched with defined supplement (6). The bacteria were collected by centrifugation (13,000 × *g*, 30 min), and the supernatant was discarded. The organisms were suspended in ethanolamine buffer (0.63 M, pH 10.5), and the pili were sheared from them with a Waring blender. The depiliated organisms and debris were then pelleted by centrifugation (13,000 × *g*, 30 min) and discarded. Pilus rods were crystallized from the supernatant by the addition of saturated ammonium sulfate in ethanolamine buffer to a final concentration of 10% (vol/vol) and collected by centrifugation. The above step was repeated until the desired purity was achieved. The purified pili, suspended in PBS (pH 6.8), were sterilized by membrane filtration (0.45-μm pore size; Millipore Corp., Bedford, Mass.) and stored in vials containing 0.01% merthiolate as a preservative. The vaccine contained 0.855 mg of pilus protein per ml as determined by UV spectrophotometry (280 nm) and was kept at 4°C until use (20).

Volunteers. Eleven female volunteers were given two intramuscular injections of either 100, 200, 500 or 1,000 μg of a gonococcal pilus vaccine 4 weeks apart. Serum and vaginal secretions were collected weekly for 8 weeks. The genital antibodies were collected on neurosurgical sponges (Welk Co., Research Triangle Park, N.C.) fashioned into vaginal tampons. The tampon was left in place for 8 to 12 h, removed, and stored in 0.9% NaCl with 0.01% merthiolate.

Three male volunteers were given a 1,000-μg intramuscular booster injection 1 year after two initial vaccinations. Four different male volunteers were given 1,000-μg intramuscular injections of a lyophilized gonococcal vaccine 4 weeks apart. Serum and seminal plasma were collected weekly for 6 weeks after vaccination. Semen was collected by masturbation and allowed to liquify, and the seminal plasma was separated by centrifugation (8,000 × *g*, 30 min).

SPRIA. The solid-phase radioimmunoassay (SPRIA; 21) was performed in polyvinyl microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.). The plates were sensitized with 100 μg of vaccine pili per ml by placing 25 μl of the suspension in each well

and incubating in a humidity chamber at 37°C for 1 h. The concentration of the antigen chosen to sensitize the plate was in the region of antigen excess. The wells were aspirated and washed once with PBS (pH 7.4) containing 1% (wt/vol) bovine serum albumin and three times with PBS (pH 7.4). Half-log dilutions of the test serum (25 μl) were added to the wells and incubated overnight in a humidity chamber at room temperature. The wells were then aspirated and washed. Twenty-five microliters of iodinated goat antihuman globulin (300 to 1,200 cpm/ng, diluted with 1% [wt/vol] bovine serum albumin to contain 20 ng of active antibody per 25 μl) was added and incubated at room temperature for 12 to 16 h. The wells were then aspirated and washed with PBS containing 1% (wt/vol) bovine serum albumin and five times with PBS. Individual wells were counted in a gamma scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). The counts per minute of ^{125}I bound was plotted versus the $\log_{10}$ of the reciprocal dilution of the primary antibody to obtain a binding curve for each serum. The linear portion of the curve was used to quantitate the amount of antibody. The antibody (micrograms per milliliter) was quantitated by using the formula of Zollinger et al. (21) which was generated by a quantitative precipitin curve.

Inhibition of epithelial cell attachment (IEA). Gonococci of transparent colony type T1 or T2 (or both) were scraped from an 18- to 20-h-old culture grown on GC medium which contained defined supplement and suspended in medium 199 (Microbiological Associates, Bethesda, Md.) supplemented with 2% (wt/vol) bovine serum albumin. The suspension was gently blended in a Vortex mixer to break up large clumps of organisms and adjusted to contain 10^7 bacteria per ml. Human buccal epithelial cells were scraped loose with a wooden applicator, suspended in PBS (pH 6.5), and washed twice with PBS. The buccal cells were enumerated in a hemocytometer and adjusted in medium 199 to a concentration of 2×10^5 cells per ml.

Buccal cells were screened for contamination and for their ability to support adhesion of the test strain before use (16). The IEA (20) was performed by mixing 50 μl of the serially diluted (twofold), heat-inactivated (56°C, 30 min) serum to be tested with 25 μl of gonococci and incubated for 30 min at 37°C on a shaker apparatus; 25 μl of a suspension of epithelial cells, adjusted so that a 50:1 ratio of organisms to epithelial cells resulted, was then added to each test tube and incubated for 30 min at 37°C. The cells were then washed in 0.15 M NaCl to remove the unattached bacteria, dried onto glass slides, and Gram stained. The total number of organisms attached to 50 buccal cells was then enumerated for each dilution and compared with controls without serum added. Since the total number of organisms attached to the control buccal cells varied each day from 450 to 1,500 organisms, the number of organisms attached to three control slides was considered as 100% attachment. The titer was arbitrarily chosen to be 50% inhibition.

Separation of immunoglobulins. Linear sucrose density gradients (10 to 30%, wt/vol) were used to separate immunoglobulins in vaginal secretions (17). Markers include 7S human immunoglobulin G (IgG) labeled with ^{125}I and 11S crystalline beet catalase (Worthington Biochemical Corp., Freehold, N.J.). Appropriate fractions were pooled and then dialyzed against 66.7 mM PBS to remove the sucrose and concentrated by using an Amicon filter apparatus (PM 10; Amicon Corp., Lexington, Mass.).

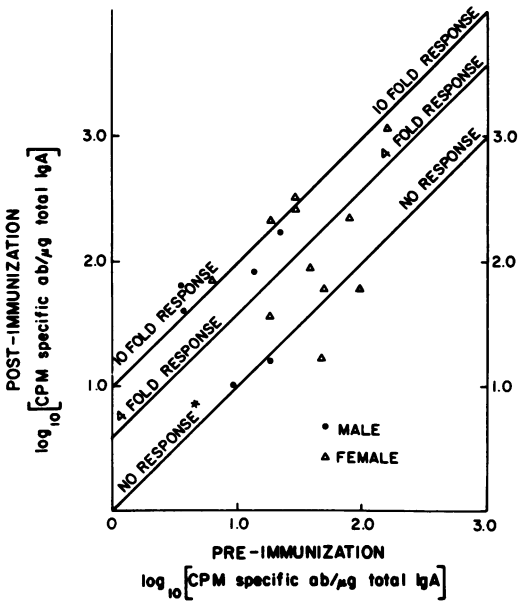

FIG. 1. Maximum fold IgA antibody rise in genital secretions as determined by SPRIA. The no-response line was generated from the preimmune levels. All points shown represent maximal fold rises (15). One male volunteer did not contribute enough secretion to determine his IgA response.

Quantitation of immunoglobulin. Immunoglobulins were quantitated in the SPRIA by using goat antihuman IgG, IgA, and IgM and secretory piece as the secondary antibody. The quantity of IgG, IgA, and IgM was calculated by the formula of Zollinger et al. (21). The secretory piece was standardized to total IgA.

RESULTS

Local genital antibody. All of the volunteers received intramuscular injections. Local genital antibodies (IgG or IgA or both) to the vaccine pili were demonstrated in the postvaccination genital washings of all of the volunteers. However, the maximal IgA fold rise was greater than four in only 9 of 17 volunteers (Fig. 1), and the maximal IgG fold rise was greater than four in 7 of 18 tested (Fig. 2). Specific antipilus IgM antibody was present in the only four male volunteers who were tested (data not shown). Thus, both men and women developed a local antibody response to a parenteral gonococcal pilus vaccine. Women tended to have higher preimmune antibody levels.

Cross-reactivity. Since the effectiveness of the vaccine depends in part upon the cross-reactivity of the antibody, the genital secretions were tested against five heterologous pilus prepara-

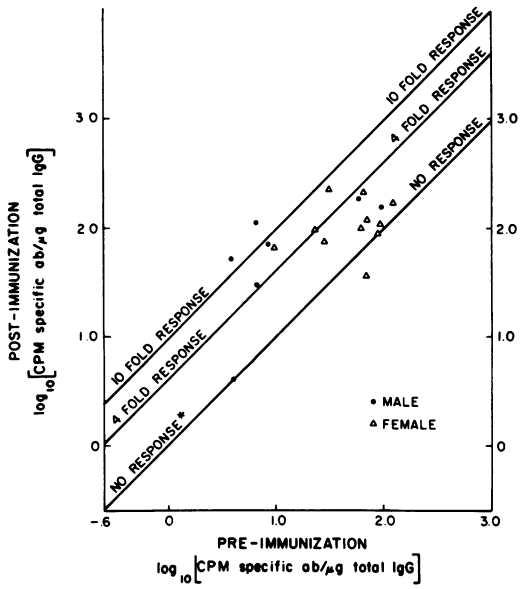

FIG. 2. Maximum fold IgG antibody rise in genital secretions as determined by SPRIA. The no-response line was generated from the preimmune levels. All points shown represent maximal fold rises (15).

tions in the SPRIA. There was an increase in antibody levels to all of the heterologous pilus preparations, but the antibody rise was dependent upon the dose given; at the 100-μg dose, cross-reactivity of the IgG class of antibody was demonstrated with only one of five heterologous pili, and the magnitude of the IgA response was markedly diminished (Table 1).

IEA. A functional activity of the genital antibody was also studied. The ability of these local secretions to block the attachment (IEA antibody) of the homologous strain to epithelial cells was present initially in 8 of 11 secretions, but increased after vaccination in only 6 of 11. Three of the five volunteers who failed to demonstrate an antibody rise had high pre-immunization titers (i.e., 1:32 or greater) (Table 2). All of the volunteers had demonstrated either a specific IgA or IgG antipilus response in the SPRIA.

Secretory (11S) antibody. The relative magnitude of the serum and genital antibody responses of 12 volunteers is shown in Fig. 3. There was a great deal of individual variation, but in general the genital antibody response tended to follow the serum antibody response, and secretions tended to follow those in the serum.

When the ratios of the mean maximal fold antibody rise of the immunoglobulin class in the secretions were compared with serum (Table 3), a disproportionate amount of IgA was found in the secretions, i.e., there was relatively more genital IgA than genital IgG. To determine what proportion of IgA was dimeric secretory (11S) IgA, the genital secretion was separated into 7S and 11S fractions on a sucrose density gradient, and the specific amount of antibody was determined for each fraction. Approximately 60% of the IgA antibody was present in the 11S fraction. However, because aggregates of IgG (and presumably of IgA) were also present and could separate into the 11S fraction, 11S-specific antipilus antibody was also determined directly with anti-human secretory piece. Specific dimeric IgA was present (Table 4).

DISCUSSION

In a previous report (20) a parenteral gonococcal pilus vaccine was shown to be safe and antigenic. All of the volunteers developed a serum immunoglobulin class-specific antibody response. The antibody was capable of blocking the attachment of gonococci to epithelial cells. A slight antibody response was also demonstrated to gonococcal lipopolysaccharide, but the antibody response for blocking attachment of gonococci was directed entirely at the pilus protein. Absorption of immune sera by a heterologous pilus reduced the inhibition of attachment antibodies to the preimmune level, suggesting that the immune response was directed at a common pilus determinant (20).

This same parenteral gonococcal pilus vaccine resulted in the production of local genital anti-

TABLE 1. Mean maximal fold antibody rise in genital secretions[a]

Dose (μg)	No. of volunteers	Pili	Fold rise in:	
			IgG	IgA
1,000	12	PGH 3-2	4.9	9.7
		174	4.0	5.3
		149	3.3	3.1
		135	2.9	3.8
		161	3.9	4.2
		418	2.7	2.7
500	3	PGH 3-2	2.7	3.0
		174	2.2	3.0
		149	2.7	3.1
		135	2.2	2.6
		161	3.1	5.0
		418	2.8	3.4
100	3	PGH 3-2	1.1	3.0
		174	1.2	<1.0
		149	≤1.0	1.2
		135	≤1.0	1.3
		161	≤1.0	1.2
		418	≤1.0	1.4

[a] The homologous and five heterologous pili were tested in the SPRIA. The fold rise was determined by dividing the post-immunization antibody level by the pre-immunization antibody level. PGH 3-2 is the homologous pilus.

TABLE 2. Inhibition of attachment of PGH 3-2 gonococci with genital secretions from volunteers immunized parenterally with PGH 3-2 gonococcus pilus vaccine

Volunteers[a]		Prepn	Inhibition titer at time (weeks) after immunization:				
No.	Sex		Pre-immunization	1–2	3–4	5–6	≥7
1	♂	Serum	1:1	1:2	1:4	1:2	
		Secretion	<1:1	<1:1	<1:1	<1:1	
2	♂	Serum	1:8	1:32		1:32	
		Secretion	<1:1	1:4		<1:1	<1:1
3	♂	Serum	1:2	1:8		1:32	
		Secretion	1:1	<1:1		<1:1	
4	♂	Serum	1:2	1:1	1:4	1:4	1:8
		Secretion	1:2	1:16			
5	♂	Serum	1:1	1:1	1:4	1:4	1:4
		Secretion	<1:1	1:32	1:16	1:32	1:1
6	♂	Serum	1:1	1:4	1:4	1:8	1:8
		Secretion	1:2	1:16	1:32	1:8	1:16
7	♀	Serum	1:4	1:4	1:8		
		Secretion	1:64	1:64	1:64	1:64	
8	♀	Serum	1:1	1:4	1:4	1:8	1:4
		Secretion	1:4	1:8	1:16	1:16	1:32
9	♀	Serum	1:2	1:4	1:4	1:4	1:4
		Secretion	1:256	1:256	1:256	1:256	1:256
10	♀	Serum	1:4	1:16	1:32	1:16	1:16
		Secretion	1:2	1:8	1:8	1:4	1:2
11	♀	Serum	1:16	1:32	1:64	1:64	1:32
		Secretion	1:32	1:32	1:32	1:32	

[a] Volunteers 1, 2, and 3 were given a 1,000-μg booster dose 1 year after the initial vaccination. Volunteers 4 through 7 were given a 1,000-μg dose of lyophilized vaccine and boosted at week 4. Volunteers 7 through 11 were given 500- to 1,000-μg doses. Volunteer 8 had known previous gonococcal infection. All of the other volunteers denied a previous gonococcal infection.

body to gonococcal pili. Women tended to have higher pre-immunization levels, and the magnitude of the response was generally greater in women than in men. Animal studies have suggested that genital antibody is under hormonal influence (9). Cervical immunoglobulin levels are known to vary during the menstrual cycle (12), and women on birth control pills have increased genital antibody levels (4). Future studies with larger numbers of human volunteers should determine whether this is a consistent difference between vaginal secretions and seminal fluid.

Specific antibody was present in all immunoglobulin classes. However, the magnitude of the IgA response was disproportionately high. At least some of the IgA was dimeric secretory (11S) IgA antibody. It cannot be determined from these studies whether specific 11S IgA was produced locally, whether serum IgA was conjugated into an 11S dimer as it was transported across the genital epithelial membrane, or whether there was a breakdown of the 7S IgG antibodies which resulted in relatively higher amounts of the more stable dimeric 11S IgA antibodies. However, there is evidence from studies in mice that IgA precursor cells selectively migrate to the genital mucosa (11) and that this migration is at least in part under hormonal influence. Furthermore, the migration of IgA precursor immunoblasts appears to be selective for mucosal tissues (2). This would argue in favor of local 11S IgA production.

There is little evidence to suggest that serum IgA is specifically transported across the mucosal barrier (2), and although the stability of dimeric 11S IgA to enzymatic digestion in the gut is well documented, the paucity of such enzymes on the genital mucosal surface would appear to preclude breakdown of monomeric IgG as an explanation of the relatively larger amounts of dimeric IgA.

The genital antibody was also found to cross-react with heterologous pili, but the extent of this cross-reactivity appeared to be dose dependent. The response was diminished at the lowest vaccine dose given, 100 μg, whereas the response was roughly the same at the two higher doses. This would suggest that there is a threshold for the production of genital antibody when a parenteral gonococcal pilus vaccine is given.

The genital antibodies were also capable of a functional activity, namely, inhibition of attachment to epithelial cells of the homologous organism. Six of 11 secretions had an antibody increase. Three of the five volunteers who failed to demonstrate an antibody increase had high prevaccination levels. All five volunteers had

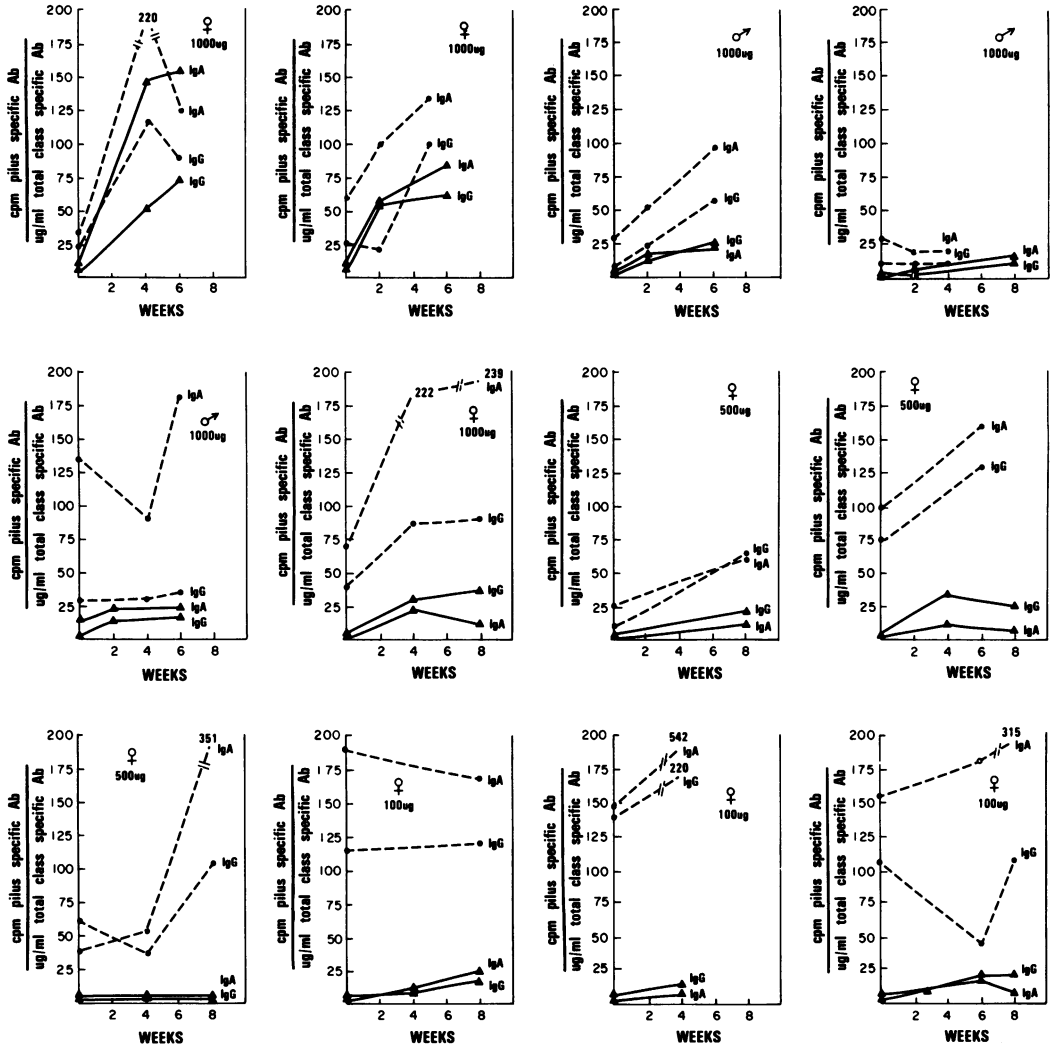

FIG. 3. Serum (▲) and genital secretory (●) antibody response to a parenteral gonococcal pilus vaccine. The total amount of both IgG and IgA in genital secretions was measured using the SPRIA with either ^{125}I-labeled antihuman IgG or ^{125}I-labeled antihuman IgA. The total class-specific antibody levels in serum were considered to be constant, and the normal values used were 13,400 and 3,900 μg/ml for IgG and IgA, respectively.

demonstrated a PGH 3-2 pilus-specific genital antibody response in the SPRIA. This would suggest that if an elevated IEA titer already exists, it is difficult to boost it with the pilus vaccine. The elevated antibody in the genital secretions without corresponding high levels in the serum are intriguing but not unique (2). Whether local stimulation before parenteral immunization or vice versa would make a difference in the amount of local antibody present must be examined in future studies. Previous studies have shown that IEA antibody is cross-reactive and directed at pilus antigen (19).

The infecting dose and the quantity of antibody will likely be important factors in protection. However, the level of antibody necessary for protection has not yet been determined.

Cross-reacting IEA activity was not examined in this study, but has previously been shown with serum antibody. Whether other functional activity of these antibodies besides IEA is present is not known. Opsonization by polymorphonuclear cells is a prominent clinical feature of the host's response. The opsonic properties of local immunoglobulins, especially dimeric 11S IgA, are controversial (10). Nevertheless, IgG theoretically would be capable of opsonization especially if active complement components C1 to C4 were present.

Antibody-dependent cytotoxicity has been de-

TABLE 3. Comparison of ratios of maximum specific antipilus IgA to maximum specific antipilus IgG in serum and genital secretions[a]

Dose (μg)	IgA/IgG ratio	
	Serum	Genital secretions
1,000	0.77	1.46
500	0.51	0.96
100	0.52	1.39

[a] The geometric mean of the maximum amount of antibody was calculated at each dose (SPRIA), and the ratios were determined as counts per minute of pilus-specific antibody per microgram of total class-specific antibody per milliliter. The concentration of total class specific antibody in serum was considered to be constant, and the normal values used were 13,400 and 3,900 μg/ml (5) for IgG and IgA, respectively. The total amount of both IgG and IgA in genital secretions was measured by using the SPRIA with either ^{125}I-labeled antihuman IgG or ^{125}I-labeled antihuman IgA.

scribed for secretory IgA, but was not examined in these studies.

The implications of these results are very encouraging. First, they indicate that induced antipilus antibodies can be found in the genital secretions. Presumably, if a vaccine for gonorrhea is to be effective, it must stimulate antibodies which will be present on the local mucosal surface. Second, the antibodies should be cross-reactive with other pili, a prerequisite if the vaccine is to be protective against heterologous strains. Third, the antibody blocks attachment of gonococci to epithelial cells. Previous studies (19) have shown that this functional antibody is related at least in part to the antipilus antibody. The protective attributes of these blocking antibodies are not yet known. These studies also offer an explanation concerning the recidivistic nature of gonococcal genital infection: little functional antibody was present 1 year after the initial vaccination (Table 2), suggesting that the response is short lived. Perhaps this problem could be circumvented by repeated local or parenteral vaccination.

The concept of immunizing with a cell wall protein to protect against a mucosal or local infection has been explored before. The results thus far have been disappointing (8). However, successful immunization against dental caries in rhesus monkeys has been reported (7), and parenteral immunization with meningococcal group C polysaccharide results in reduced meningococcal group C carriage in humans (1). Also, an *Escherichia coli* pilus vaccine has protected piglets given colostrum from sows parenterally immunized (11, 13). Thus, taken as a whole, these results suggest that a parenteral gonococcal pilus vaccine may be effective in preventing mucosal infection by blocking the initial step in pathogenesis, namely, the attachment of gonococci to mucosal epithelial cells.

TABLE 4. Specific IgA antibodies in genital secretions[a]

Volunteer	Secretion	cpm of specific antibody per μg of total IgA in secretion	
		IgA	Secretory piece
2	Pre-immunization	28.3 ± 6.5	7.5 ± 2.6
	Post-immunization	59.7 ± 12.7	15.4 ± 3.2
3	Pre-immunization	3.9 ± 0.4	1.7 ± 0.4
	Post-immunization	7.9 ± 0.6	2.1 ± 0.3
5	Pre-immunization	17.3 ± 1.1	2.2 ± 0.9
	Post-immunization	73.6 ± 8.3	7.1 ± 1.7
7	Pre-immunization	6.0 ± 3.9	3.0 ± 0.3
	Post-immunization	179.3 ± 27.3	39.4 ± 3.6
9	Pre-immunization	5.8 ± 0.5	2.0 ± 0.7
	Post-immunization	12.6 ± 8.0	8.2 ± 3.4
10	Pre-immunization	16.5 ± 3.4	4.6 ± 1.6
	Post-immunization	4.2 ± 1.4	3.5 ± 0.9

[a] The SPRIA was used to determine IgA and 11S IgA levels. Goat antihuman secretory piece antibody was used to estimate the levels of specific dimeric 11S IgA. Volunteers 2, 3, and 5 were male: volunteers 7, 9, and 10 were female. Quadruplicate samples were run.

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LITERATURE CITED

1. **Artenstein, M. S., R. Gold, J. G. Zimmerly, F. A. Wyle, H. Schneider, and C. Harkins.** 1970. Prevention of meningococcal disease by group C polysaccharide vaccine. New Engl. J. Med. **282**:417–420.
2. **Bienenstock, J., and A. D. Befus.** 1980. Mucosal immunology. Immunology **41**:249–267.
3. **Brinton, C. C., J. Bryan, J. Dillon, N. Guerina, L. J. Jacobson, A. Labik, S. Lee, A. Levine, S. Lim, J. McMichael, S. Polen, K. Rogers, A. C.-C. To, and S. C.-M. To.** 1978. Uses of pili in gonorrhea control: role of bacterial pili in disease, purification and properties of gonococcal pili, and progress in the development of a gonococcal pilus vaccine for gonorrhea, p. 155–178. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.

3a. **Brinton, C. C., S. W. Wood, A. Brown, A. M. Labik, J. R. Bryan, S. W. Lee, S. E. Polen, E. C. Tramont, J. Sadoff, and W. Zollinger.** 1982. The development of a neisserial pilus vaccine for gonorrhea and meningococcal meningitis, p. 140–159. *In* J. Robbins, J. Hill, and J. Sadoff (ed.), Seminars in infectious disease, vol. IV. Thieme-Stratton, New York.

4. **Chipperfield, E. J., and B. A. Evans.** 1975. Effect of local infection and oral contraception immunoglobulin levels in cervical mucus. Infect. Immun. **11**:215–221.
5. **Eisen, H. N.** 1980. Immunoglobulin molecules and genes, p. 378. *In* B. D. Davis, R. Dulbecco, H. N. Eisen and H. S. Ginsberg (ed.), Microbiology. Harper & Row, Publishers, New York.

6. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. Pirkle.** 1963. I. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85**:1274–1279.
7. **Lehner, T., S. J. Challaumbe, and J. Caldwell.** 1980. Oral immunization with streptococcus in rhesus monkeys and the development of immune responses and dental caries. Immunology **41**:857–864.
8. **McDermott, M. R., and J. Bienenstock.** 1979. Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory and genital tissues. J. Immunol. **122**:1892–1898.
9. **McDermott, M. R., D. A. Clark, and J. Bienenstock.** 1980. Evidence for a common mucosal immunologic system. II. Influence of the estrous cycle on B immunoblast migration into genital and intestinal tissues. J. Immunol. **124**:2536–2539.
10. **McMillan, A., G. McNeillage, and H. Young.** 1979. Antibodies of *Neisseria gonorrhoeae*: a study of the urethra exudates of 232 men. J. Infect. Dis. **140**:89–95.
11. **Morgan, E. L., R. E. Isaacson, H. W. Moon, C. C. Brinton, and C. C. To.** 1978. Immunization of suckling pigs against enterotoxigenic *Escherichia coli*-induced diarrheal disease by vaccinating dams with purified 987 or K99 pili: protection correlates with pilus homology of vaccine and control. Infect. Immun. **22**:771–777.
12. **Mossman, H. W.** 1973. Soluble protein in cervical mucus, p. 201–233. *In* R. J. Blandar and K. Moghissi (ed.), The biology of the cervix. University of Chicago Press, Chicago.
13. **Nagy, B., H. W. Moon, R. E. Isaacson, and C. C. Brinton.** 1979. Immunization of suckling pigs against enteric enterotoxigenic *Escherichia coli* infection by vaccinating dams with purified pili. Infect. Immun. **21**:269–274.
14. **Pearce, W. A., and T. A. Buchanan.** 1978. Attachment role of gonococcal pili. J. Clin. Invest. **61**:931–943.
15. **Siber, G. R., B. J. Ransil, and G. Schiffman.** 1980. Graphical method for evaluating antibody response to vaccine. Infect. Immun. **28**:641–644.
16. **Tramont, E. C.** 1976. Specificity of inhibition of epithelial cell adhesion of *Neisseria gonorrhoeae*. Infect. Immun. **14**:593–595.
17. **Tramont, E. C.** 1977. Inhibition of adherence of *Neisseria gonorrhoeae* by human genital secretions. J. Clin. Invest. **59**:117–124.
18. **Tramont, E. C., and J. Ciak.** 1978. Antigonococcal antibodies in genital secretions, p. 274–278. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
19. **Tramont, E. C., J. Ciak, J. Boslego, D. G. McChesney, C. C. Brinton, and W. Zollinger.** 1980. Antigenic specificity of antibodies in vaginal secretions during infection with *Neisseria gonorrhoeae*. J. Infect. Dis. **142**:23–31.
20. **Tramont, E. C., J. C. Sadoff, J. W. Boslego, J. Ciak, D. G. McChesney, C. C. Brinton, S. Wood, and E. Takafuji.** 1981. Gonococcal pilus vaccine: studies of antigenicity and inhibition of attachment. J. Clin. Invest. **68**:881–888.
21. **Zollinger, W. D., J. M. Dalrymple, and M. S. Artenstein.** 1976. Analysis of parameters affecting the solid phase radioimmunoassay quantitation of antibody to meningococcal antigens. J. Immunol. **117**:1788–1789.