Gonococcal Pilus Vaccine

STUDIES OF ANTIGENICITY AND INHIBITION OF ATTACHMENT

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ABSTRACT A gonococcal pilus vaccine or placebo was injected subcutaneously or intramuscularly into 71 human volunteers. The vaccine was found to be safe. The principal adverse reaction was a complaint of a sore arm, which was caused, at least in part, to the volume of material injected. 6 of 64 (9%) volunteers receiving the larger doses also complained of malaise. The vaccine was found to be antigenic. All of the volunteers developed an immunoglobulin class-specific antibody response as measured by a solid phase radioimmunoassay. 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 responsible for blocking attachment of gonococci was directed entirely at the pilus protein. The stimulated antibodies were shown to crossreact with isolated pili of heterologous gonococcal strains and to block the attachment of heterologous gonococci. Absorption of immune sera by a heterologous pilus reduced the inhibition of attachment antibodies to the pre-immune level, suggesting that the immune response was directed at a common pilus determinant.

INTRODUCTION

Gonorrhea is the most frequently reported communicable disease in the United States. Over 1 million cases were reported in 1979 and it is estimated that at least an equal number of cases were not reported (1). The methods of education, contact tracing, and early treatment presently used to control the spread of gonorrhea have proved not to be entirely effective. Furthermore, gonococcal pelvic inflammatory disease or salpingitis is associated with a significant incidence of recurrence, infertility, and ectopic pregnancy (2, 3). An effective vaccine would offer promising additional means of controlling this illness.

The description of virulent colony types of gonococci by Kellogg and his colleagues (4) provided a major clue to the pathogenesis of gonorrhea. The most striking difference in the organisms making up the virulent colonies was the presence of pili (5).

Gonococcal pili are protein filaments of ~20,000 daltons that extend out from the cell wall (6). They have been shown to be the principle mediators of attachment of gonococci to mammalian cells (6–8), may contribute to the organisms ability to resist phagocytosis (9–15), and may be involved in surface translocation by twitching motility (6). Antibodies to pili have been demonstrated in patients with gonorrhea (16) and they have been shown to block the attachment of gonococci to human mucosal cells (17).

Pili have been isolated and purified as a potential vaccine candidate (6, 18) and in preliminary studies in a limited number of volunteers have been shown to be safe and antigenic (6, 19). This report describes the safety and antigenicity of a prototype gonococcal pilus vaccine, and demonstrates the ability of these antibodies to inhibit attachment of gonococci to buccal epithelial cells, a possible mechanism by which these antibodies might function to protect the host (17, 18).

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METHODS

Gonococcal pilus vaccine. The gonococcal pilus vaccine (Food and Drug Administration, Investigational New Drug Application 1351) was prepared at the University of Pittsburgh by Charles C. Brinton, Ph.D.[1] (20) from gonococcal strain Pgh 3–2, originally isolated from the urethra of an infected male patient. Cloned piliated phase type 2 colonies of gonococci were harvested into phosphate-buffered saline (PBS) (pH 6.8) from the surface of solid GC medium (Difco Laboratories, Detroit, Mich.) enriched with defined supplement (4). The bacteria were collected by centrifugation (13,000 *g* for 30 min), and the supernate discarded. The organisms were resuspended in ethanolamine buffer (0.63 M, pH 10.5), and the pili sheared from them with a Waring blender (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn.). The depiliated organisms were then sedimented by centrifugation (13,000 *g* for 30 min). The shearing and centrifugation steps were repeated and the pelleted organisms discarded. Pilus rods were crystallized from the supernate by the addition of 10% (vol/vol) saturated ammonium sulfate in ethanolamine buffer. Pili were collected by centrifugation. The purified pili, suspended in 0.01 M PBS (pH 6.8), were sterilized by membrane filtration (0.045 μm millipore filters, Millipore Corp., Bedford, Mass.) and stored in vials containing 0.01% merthiolate as a preservative. The vaccine contained 0.855 mg/ml of pilus protein as determined by UV spectrophotometry (280 nm) and was kept at 4°C until use (19).

Criteria for purity. The criteria for purity of the vaccine preparation (Investigational New Drug Application 1351) were darkfield microscopy, electron microscopy, sodium dodecyl sulfate, polyacrylamide gel electrophoresis, UV absorption spectra and amino acid analysis. The tests were performed at the University of Pittsburgh. Darkfield microscopy revealed the presence of pilus crystals and the absence of detectable bacterial cells or other large particulates. Electron microscopy revealed only pilus crystals, single pili and a very small amount of pilus substructures and electron dense material. The ultraviolet spectrum was similar to that found with previous preparations of Pgh 3–2 pili (6). A single main band of Coomassie Blue staining material was seen in the sodium dodecyl sulfate polyacrylamide gel at the expected position for *N. gonorrhoeae* Pgh 3–2 pili. When the appropriate plot was made of the positions of the standard proteins (bovine serum albumin: 68,000 daltons, ovalbumin: 43,000 daltons, papain: 23,000 daltons, myoglobin: 17,600 daltons, cytochrome *c*: 12,400 daltons) the molecular mass of the vaccine pilus band was determined to be 20,800 daltons. A very minor band at 40,300 daltons was also observed on the more heavily loaded gels. Densitometer tracings of the gel patterns show that this band compromised ~3% of the total protein. It was felt that this band was a gonococcal pilin dimer since it appeared close to the position that would be expected for a dimer of GC pilin resulting from the alkaline conditions used in the purification and filtration of the vaccine. The amino acid analysis of the vaccine pili was sufficiently distinctive to allow it also to be used as a measure of purity and identity.

A quantitative limulus lysate assay was kindly performed for us by Robert Seid, Ph.D., Walter Reed Army Institute of Research, using a highly purified standard *Escherichia coli* lipopolysaccharide (LPS)[2] preparation. The gonococcal vaccine jelled the limulus lysate at an equivalence of 10.45 ng/ml of the standard *E. coli* endotoxin (LPS).

Placebo. A placebo vaccine was manufactured by following all of the same procedures as for the pilus vaccine except that no organisms were inoculated onto the culture trays.

Volunteers. The first nine volunteers were healthy professional (M.D., Ph.D) male laboratory personnel from the Walter Reed Army Institute of Research (mean age, 38.9 yr). The second group of 62 volunteers were healthy military medical field personnel assigned to Ft. Bragg, North Carolina. They included 45 men and 17 women (mean age, 27.3 yr).

All of the volunteers were screened for *N. gonorrhoeae* with a culture of their urethra or cervix upon entry into the study. No volunteer had an active gonococcal infection detected at the time of immunization. All of the women volunteers had a normal pelvic examination and a negative pregnancy test. Six of the volunteers gave a history of having had gonorrhea sometime in the past.

All of the volunteers for the study were informed of the potential hazards in compliance with the standards of the U. S. Public Health Service, of the Human Use Committee of the Walter Reed Army Institute of Research, and the Office of the Surgeon General of the U. S. Army in accordance with U. S. Army Regulation (AR40-7).

Dosage and route. The number of volunteers receiving the various vaccine and placebo dosages are shown in Table I. Each volunteer received two injections 4 wk apart. The initial 9 laboratory volunteers were inoculated subcutaneously with either a 1,000 μg (7 volunteers) or a 2,000 μg dose (2 volunteers), while the 62 field volunteers were all immunized intramuscularly. The field volunteers were randomized in a double-blind fashion to receive a 100, 200, 500 or 1,000 μg dose of vaccine or placebo. The volume of material given varied for each dose (Table I). Individuals who received a placebo vaccine were randomized within each dose.

Volunteer follow-up. After each injection the initial nine laboratory volunteers were examined, interviewed and completed a questionnaire regarding adverse reactions. Oral temperatures were obtained every 6–8 h during the immediate 24–48 h postimmunization. A CBC, SMAC-20, and urinalysis were also performed for the immediate 4 successive wk following each immunization.

The field volunteers were observed for 15–30 min after receiving each injection. They were also personally interviewed and/or given questionnaires for recording any side effects. Of the original 62 volunteers, 57 received a second injection. One volunteer refused a second injection. The four other volunteers who did not receive a second injection had either been transferred to another duty station or were on leave status.

Antibody studies

Solid-phase radioimmunoassay (SPRIA) (20). SPRIA was performed in polyvinyl microtiter plates. The plates were sensitized with 25 μl of a 100 μg/ml concentration of vaccine pili or 50 μg/ml concentration LPS and placed 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 BSA 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

[1] Brinton, C. C., S. Wood, J. Bryan, A. Labik, S. Polen, H. Brown, K. Rogers, N. Guerina, E. C. Tramont. 1981. Preparation, testing, safety, antigenicity, immunogenicity and serological specificity of a purified gonococcal pilus vaccine. Manuscript in preparation.

[2] *Abbreviations used in this paper*: BSA, bovine serum albumin; IEA, inhibition of epithelial cell attachment; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; SPRIA, solid-phase radioimmunoassay.

at room temperature. The wells were then aspirated and washed. 25 μl of iodinated (^{125}I) goat antihuman globulin (200–1,200 cpm/ng diluted with 1% BSA to contain 20 ng of active antibody per 25 μl) was added and incubated at room temperatures for 12–16 h. The wells were then aspirated and washed with PBS containing 1% wt/vol BSA and four times with PBS. Individual wells were counted in a Gamma Scintillation Counter (Beckman Instruments, Inc., Palo Alto, Calif.). The counts per minute of ^{125}I bound were plotted vs. 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. Quantitation of antibody (micrograms per milliliter) was achieved by reference to the formula described by Zollinger (21) which was generated by a quantitative precipitin curve.

Inhibition of epithelial cell attachment (IEA) (17). Gonococci of transparent colony type T1 and/or T2 were scraped from an 18–20-h culture grown on GC medium (Difco Laboratories) which contained defined supplement, and suspended in Medium 199 (Microbiological Associates, Walkersville, Md.), supplemented with 2% wt/vol BSA. The suspension was gently vortexed to break up large clumps of organisms and adjusted to contain 1×10^7 bacteria/ml (determined with a Petroff-Houser bacteria counter obtained from C. A. Hausser & Son, Philadelphia, Pa.). Human buccal epithelial cells were scraped loose with a wooden applicator, suspended in PBS, pH 4.5, 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/ml.

Buccal cells were screened for contamination and for their ability to support adhesion of the test strain prior to use (22). The IEA was performed by mixing 0.05 ml of the serially diluted (twofold) heat inactivated (56°C for 30 min) serum with 0.025 ml of gonococci and incubated for 30 min at 37°C on a shaker apparatus; 0.025 ml of a suspension of epithelial cells was then added to each test tube and reincubated for 30 min at 37°C. The organism to epithelial cell ratio was 50:1. The cells were washed in 0.15 M NaCl to remove the unattached bacteria, dried onto glass slides and stained by Gram's method. The total number of organisms attached to 50 buccal cells were enumerated for each dilution and compared to controls without serum added. Because the total number of organisms attached to the control buccal cells varied each day from 450–1,500 organisms, the amount of inhibition was expressed in terms of percent of inhibition. The average number of organisms attached to three control slides was considered as 100% attachment. The titer was arbitrarily chosen at 50% inhibition (17).

Serum adsorption (17). The LPS was bound to alum by mixing 1 mg of LPS/ml with 0.9 ml of 0.05 M aluminum hydroxide gel (0.05 M $AlCl_3$ in NaOH, pH 6.6) at room temperature for 30 min. The LPS-alum mixture was then incubated with an equal volume of 1% (wt/vol) BSA solution for 2 h at 37°C to bind the BSA to nonspecific sites on the alum. The LPS-alum complex was sedimented by centrifugation (12,000 *g* for 20 min) and washed twice with 0.15 M NaCl.

Prior to adsorption, the antigens were pelleted by centrifugation (purified pilus crystals, 13,000 *g* for 60 min; and LPS bound to alum, 12,000 *g* for 20 min). The pelleted antigen (pili, 0.050 mg; LPS-alum, 1 mg) was resuspended in 0.5 ml of serum. The antigen-serum mixture was incubated on a shaker apparatus at 37°C for 60 min and then at 4°C for 60 min. The antigen-antibody complex was removed by centrifugation as described above.

The efficiency of the adsorption of gonococcal antibody was determined by the SPRIA. Specific antigens were added until the amount of antibody in the adsorbed secretion that bound to a specific gonococcal antigen had reached base-line levels.

Serum fractionation. Serum was fractionated into IgG, IgA, and IgM immunoglobulin classes by batch adsorption with immunobeads (Bio-Rad Laboratories, Richmond, Calif.). The efficiency of each adsorption was monitored with low level immunoprecipitin plates (Partigen plates, Behring Diagnostics, American Hoechst Corp., Somerville, N. J.).

Rabbit immunization. Two 1.2 kg New Zealand albino rabbits were immunized in the footpad with 0.855 mg pilus vaccine plus Freund's adjuvant, followed by two additional subcutaneous injections without Freund's adjuvant at 1 and 5 wk. Hyperimmune sera was obtained after 42 d.

LPS. Purified LPS was extracted from the Pgh 3–2 gonococci by the phenol-water method of Westphal et al. (23). Protein contamination was tested for by use of the method of Lowry et al. (24); nucleic acid contamination was monitored by ultraviolet spectrophotometry (25); 2-keto-3-deoxy sugar concentration was determined by use of the method of Osborne (26).

RESULTS

Adverse reactions. The gonococcal vaccine was shown to be safe. 64 of the 71 volunteers (89%) returned the questionnaires or were interviewed within 24 h of the initial vaccination. Soreness at the site of injection was the most common complaint and occurred in 50 of

TABLE I

Number of Laboratory and Field Volunteers Receiving Each Dose of Vaccine

	Dose and volume					
	100 μg 0.12 ml	200 μg 0.24 ml	500 μg 0.60 ml	1,000 μg 1.2 ml	2,000 μg 2.4 ml	Totals
Vaccine						
F	3	3	5	4	0	15
M	11	11	9	15	2	48
Placebo						
F	0	1	0	1	0	2
M	1	1	3	1	0	6
Totals	15	16	17	21	2	71

F, Female; M, Male.

TABLE II
Antibody Response to Pgh 3–2 Gonococcal Pilus Vaccine

Wk	IgG	IgM	IgA
		μg/ml	
0	1.0±0.1	2.5±2.0	0.9±0.3
1	10.0±10.1	60.3±78.0	11.1±14.4
2	20.4±11.4	40.4±22.5	20.7±20.5
3	17.5±8.3	33.2±32.4	16.0±15.8
4	16.4±8.7	25.8±20.2	9.7±11.4
5	22.4±4.1	22.7±20.8	10.4±7.6
6	27.8±3.5	24.2±17.4	7.1±4.2
8	25±4.3	12.2±4.3	4.0±2.1
15	17.1±8.3	7.5±3.9	2.1±2.2
30	11.0±4.4	5.1±2.2	1.5±1.0

The mean and SD of the serum antibody response in the seven laboratory volunteers who received two 1,000-μg doses was determined using the SPRIA. The booster dose was given after the 4th wk bleeding. The variation (SD) on individual responses after the booster dose was less marked than after the initial dose.

64 volunteers (90%) who had received the vaccine. However, five of seven volunteers who had received the placebo vaccination and were interviewed also experienced this side effect. All five had received either 0.65 ml or 1.3 ml of the placebo vaccine which suggested that this reaction was related at least in part to the volume of material given. 6 of 33 who had received 500 μg or more (0.60–2.4 ml) of the vaccine also reported malaise. Most of the adverse reactions passed by 24 h and none lasted longer than 96 h. 66% of the volunteers complained of a sore arm after the second vaccination and one volunteer recorded a single temperature of 100.2°F 6 h after being immunized. No serious side effects were observed after either vaccination, and no person requested that he or she be excused from duty. The values for the CBC, SMAC-20, and urinalyses performed weekly for 8 wk on the initial nine volunteers remained within normal limits.

Antibody studies. The gonococcal pilus vaccine was shown to be antigenic. Class specific antipilus antibodies determined by SPRIA on the initial laboratory volunteers who had received 1,000 μg doses is shown in Table II. There was marked variation among the volunteers in the amount of specific antipilus antibody stimulated but this difference lessened with the booster vaccination. Five of these seven volunteers reached their peak IgM and IgA levels after the initial vaccination, while five of seven volunteers reached their IgG peak after the booster vaccination. Serum antipilus antibody, primarily IgG, was still present at 30 wk. The mean maximum serum antibody level and the median maximum antibody fold increase in these same volunteers, is shown in Table III.

Because of the recognized difficulty in the complete and absolute separation of bacterial cell wall proteins from LPS, the large amount of vaccine given, and the results of the limulus lysate that suggested LPS contamination, antibody to LPS was measured directly using the SPRIA. Two rabbits were hyperimmunized with the Pgh 3–2 pilus vaccine. The mean pre-immunization antipilus LPS antibody level was 0.53 μg/ml and the 6-wk mean post-immunization level was 5.6 μg/ml which constituted a 10-fold rise, while the mean pre-immunization antipilus antibody level of 0.34 μg/ml and the 6-wk post-immunization level of 660 μg/ml which constituted a 1,320-fold antibody rise. The seven human laboratory volunteers who had received 1,000-μg vaccine doses were also tested. They were found to have had an average of 0.42 μg/ml of pre-vaccination anti-LPS antibody and a mean peak of 0.62 μg/ml of post-vaccination anti-LPS antibody at 2 wk, which is a small antibody increase. However, all of the volunteers had an increase in anti-LPS antibody suggesting an immunologic response, albeit slight, to the contaminating LPS (Table IV). These small antibody increases were of the IgG immunoglobulin class only. Because this slight antibody increase may have been due to a nonspecific polyclonal antibody response, competitive

TABLE III
Mean Maximum Serum Antipilus Antibody Level and Median Maximum Antibody Fold Increases in Seven Laboratory Volunteers Who Received 1,000-μg Doses

	IgG	IgM	IgA
Mean maximum serum antibody level, *μg/ml*	27.5±7.2 (20.5–40.0)	62.7±75 0 (12.8–193.0)	21.1±18.5 (4.9–56.0)
Median maximum fold increase	67.2 (45.0–93.0	15.8 (11.0–107.0)	60.0 (25.0–280.0)

A booster vaccination was given at 4 wk. The antibody was determined by the SPRIA. The maximum antibody level was always reached within the first 8 wk. The ranges are shown in parentheses.

TABLE IV

Antibody Responses to Purified Pgh 3–2 LPS in Seven Laboratory Volunteers

Volunteer	IgG Anti-LPS antibody							
	Pre-imm	2 wk	4 wk	6 wk	8 wk	16 wk	30 wk	40 wk
				μg/ml				
1	0.41	0.53	0.48	0.57	0.60	0.50	0.42	0.30
2	0.33	0.65	0.60	0.50	0.58	—	0.54	0.44
3	0.38	0.44	0.39	0.41	0.68	0.64	0.56	0.52
4	0.78	0.89	—	0.87	0.68	0.62	0.62	0.48
5	0.18	0.30	0.30	0.30	0.42	0.46	0.38	—
6	0.48	0.81	0.63	0.66	—	0.46	0.40	0.34
7	0.40	0.73	0.67	0.58	0.42	—	0.42	0.36
Mean	0.42	0.62	0.51	0.56	0.56	0.54	0.48	0.41

SPRIA was used to determine the amount of antibody to LPS. The booster vaccination was given after the 4th wk bleed. All volunteers had received a 1,000-μg dose. All volunteers had a slight increase in antibody to Pgh 3–2 LPS. Volunteers given 1,000-μg doses were studied in order to insure a maximum response to LPS. Pre-imm, pre-immunization.

inhibition of the anti-LPS antibody was done using purified Pgh 3–2 gonococcal LPS. The antibody binding was blocked and therefore was shown to be specific for the vaccine strain of LPS.

The antisera were also assayed for pilus specific antibodies against three heterologous pili to determine the degree of crossreactivity. Individuals from the different dosage groups were randomly chosen and the geometric mean fold rise was calculated (Table V). Every individual had a specific rise to each heterologous pili but the individual variation was great. The range tended to lessen after the booster dose was given and

TABLE V

Geometric Mean Fold Rise against the Homologous and Three Heterologous Pili

Dose	Number	Pili	IgG		IgA	
			2 wk	6 wk	2 wk	6 wk
100 μg	7	Pgh 3–2	10.2±4.3	14.1±2.8	13.0±4.5	10.6±2.9
		135	5.0±4.1	4.7±2.6	4.4±5.3	5.4±2.9
		149	6.0±4.2	5.9±3.2	3.0±6.2	2.5±4.2
		161	7.0±4.8	6.8±2.9	4.3±5.6	3.9±3.6
200 μg	7	Pgh 3–2	6.9±2.0	7.0±1.8	12.7±1.8	6.7±1.3
		135	3.4±1.6	3.2±1.3	4.6±3.2	2.6±3.0
		149	4.1±1.8	3.6±1.6	1.7±1.4	1.2±1.4
		161	3.5±1.9	3.0±1.8	3.6±1.8	2.3±1.9
500 μg	6	Pgh 3–2	23.9±2.5	13.9±2.1	21.3±3.9	9.2±3.3
		135	9.3±1.9	5.0±1.4	6.5±1.8	3.2±1.6
		149	13.3±2.4	5.7±2.0	4.0±2.3	2.4±2.3
		161	12.9±3.3	6.4±2.6	3.8±2.3	1.8±1.6
1,000 μg	7	Pgh 3–2	15.0±1.9	8.6±1.5	26.9±1.6	9.7±1.7
		135	7.5±1.6	4.2±1.9	5.4±2.8	1.9±1.7
		149	13.3±2.0	4.9±2.6	3.8±2.9	2.0±1.9
		161	14.4±1.8	6.1±1.6	3.9±1.9	2.2±1.9

2- and 6-wk post-immunization sera were tested in the SPRIA. The booster vaccination was given at the 4th wk. The number of individuals in each group were chosen at random. Pgh 3–2 pili were the homologous vaccine pili.

TABLE VI
IEA of Vaccine Strain

Vol	Weeks								
	Pre-imm	1	2	4	6	8	16	30	40
1	1:4	1:16	1:32	1:32	1:32	1:16	1:32	1:32	1:16
2	1:1	1:2	1:8	1:8	1:4	1:8	1:8	1:4	1:2
4	<1:1	1:8	1:16	1:8	1:16	1:32	1:16	1:8	1:8
5	1:2	1:2	1:8	1:8	1:8	—	1:8	1:8	1:16
9	1:1	1:8	1:16	1:16	1:16	1:16	—	1:16	—
25	1:1	—	1:4	1:4	1:8	1:4	—	—	—
28	1:4	—	1:16	1:32	1:16	1:16	—	—	—
30	1:16	1:32	—	1:64	1:64	1:32	—	—	—
31	1:1	1:4	—	1:4	1:4	1:4	—	—	—

A booster vaccination was given after the 4th wk sample was obtained. Volunteers 1 and 2 received 2,000 μg doses. Volunteers 4, 5, 9, 25, and 28 received 1,000-μg doses, Volunteer 30 received a 200-μg dose, Volunteer 31 received a 100-μg dose. Volunteers 1 and 30 had had a previous gonococcal infection. The volunteers studied were randomly chosen. Volunteers 1–9 were male. Volunteers 25, 28, 30, and 31 were females. Pre-imm, preimmunization.

the antibody fold increase to heterologous pili was found to be less effected by the dose given than for the homologous vaccine pilus.

Sera from nine volunteers were tested for their ability to inhibit epithelial cell attachment. All of the volunteers studied developed an increase in their IEA antibody titer to the vaccine strain (Table VI). The serum of one of the volunteers was fractionated into IgG, IgA, and IgM immunoglobulin classes and all classes of the antibody were found to inhibit attachment.

Because a slight amount of antibody was raised to LPS, anti-LPS antibody was examined for its ability to inhibit attachment. Post-vaccination sera (volunteer 4, 4th wk) was adsorbed with purified Pgh 3–2 LPS and vaccine pili. The IEA titer was uneffected by adsorption with LPS, while adsorption with the vaccine pili reduced the IEA titer to its preimmunization level (Table VII). Adsorption with LPS slightly reduced the amount of antibody binding to the vaccine pilus preparation.

TABLE VII
Absorption of Postvaccination Sera with Purified Pgh 3–2 LPS and Pgh 3–2 Pili

Serum	Anti-LPS antibody	Anti-pilus antibody	IEA titer
	μg/ml		
Pre-imm	0.51	0.56	<1:1
Post-imm unadsorbed	0.75	17.41	1:16
Post-imm adsorbed LPS	0.59	16.93	1:32
Post-imm adsorbed pili	0.41	0.48	<1:1

Serum from volunteer 4 (1,000-μg dose) was adsorbed with Pgh 3–2 LPS. An aliquot was then adsorbed with vaccine pili. Antibody to LPS and pili were determined using the SPRIA. Pre-imm, preimmunization; Post-imm, postimmunization.

Because of the importance of crossreactivity against heterologous strains, sera from three volunteers were tested for IEA antibody rises against eight heterologous strains. An increase in IEA antibody titer was demonstrated against all of the heterologous strains (12 determinations). However, half of the titer rises (six) were of a small magnitude (Table VIII).

To determine whether the IEA antibody against the heterologous pili might be directed at a common determinant(s) on each pilus strain, sera was adsorbed with one heterologous pili and tested against different strains. Adsorption reduced the IEA titer against all three of the heterologous strains to pre-immunization levels (Table IX).

DISCUSSION

The Pgh 3–2 gonococcal pilus vaccine was shown to be safe and well tolerated. No serious adverse reactions occurred. The most common complaint was that of a sore arm and this correlated with the volume of material injected, although subjectively, the degree of soreness appeared to be greatest in those receiving the vaccine.

The Pgh 3–2 gonococcal pilus vaccine was found to be antigenic. Parenteral administration of the vaccine produced a serum antibody response in the three principal immunoglobulin classes in all volunteers tested with the SPRIA. The antibody rise to the homologous pili generally correlated with the amount of antigen

TABLE VIII

Inhibition of Attachment of Heterologous Strains by Serum from Three Volunteers Who Had Received Two 1,000-μg Doses of Vaccine

Week	Vaccine strain	Heterologous strains 1	2	3	4	5	6	7	8
Pre-imm	1:1	<1:1	1:1	<1:1	1:1	ND	ND	ND	ND
4 wk post	1:8	1:1	1:2	1:4	1:4	ND	ND	ND	ND
Pre-imm	1:1	1:1	1:1	ND	ND	1:1	1:1	ND	ND
7 wk post	1:8	1:2	1:2	ND	ND	1:4	1:4	ND	ND
Pre-imm	1:2	ND	ND	1:2	1:2	ND	ND	1:1	1:1
7 wk post	1:16	ND	ND	1:8	1:4	ND	ND	1:8	1:2

Pre-imm = preimmunization sera, Post = number of wk post-immunization volunteer was bled. Serum and strains were chosen at random. ND, not done.

(pilus vaccine) given. The booster vaccination tended to raise the antibody level of the low responders but did not appreciably boost those who had had a good initial response.

The antipilus antibody was also capable of functional activity, namely IEA. It is theorized that this is at least one of the means by which this antibody might function to protect the host (17, 18, 27). All of the volunteers tested demonstrated an IEA titer rise to the vaccine strain. Furthermore, an IEA antibody titer rise was demonstrated in 12 post-vaccination sera against eight heterologous strains. The titers were always lower than that demonstrated against the vaccine strain. However, the correlation of IEA antibody with immunity must be determined in future field studies.

The Pgh 3–2 gonococcal vaccine contained a minute amount of LPS as demonstrated in the anti-LPS antibody rise in hyperimmunized rabbits. The seven human volunteers tested also developed an anti-LPS antibody rise, but of very low magnitude. However, adsorption with purified LPS failed to reduce the IEA antibody. Thus, the induced antibody that inhibited attachment was directed entirely at the pilus protein.

There was an antibody increase to the three heterologous pili tested. This supports the results of previous studies which demonstrated that adsorption with one heterologous pilus preparation removed all of the crossreactive antibody to other heterologous pili (6). Furthermore, all of the crossreactive IEA antibody in one serum was also removed by adsorption with a heterologous pilus, suggesting that the crossreactive pilus antibody is directed at a common determinant(s) (Table IX).

The recidivistic nature of gonococcal infections would suggest that immunity to gonorrhea does not always develop. There is evidence to suggest that this may be due to a poor immune response, a short-lived immune response and/or antigenic variation among strains of gonococci. However, this should not discourage attempts to stimulate an effective immune response by vaccination as a possible means of controlling this disease. Furthermore, cross reacting antibody to heterologous gonococcal pili and gonococcal strains, a prerequisite for vaccine efficacy, has been demonstrated. A prototype *E. coli* pilus vaccine has been shown to protect piglets that had been given colostrum from sows parenterally immunized with *E. coli* pili (28, 29). The efficacy of the gonococcal pilus vaccine must await clinical field trials.

TABLE IX

Adsorption of Post-immunization Sera with Heterologous Pili

Sera	SPRIA Pgh 3-2	SPRIA 161	IEA titer Pgh 3-2	IEA titer 1	IEA titer 4
	μg/ml				
Pre-imm	1.22	1.61	1:2	1:2	1:1
Post-imm	29.70	22.95	1:16	1:4	1:4
Post-imm adsorbed with 161 pili	5.32	0.61	1:2	1:1	1:1

Post-immunization sera was adsorbed with heterologous pili 161 (Methods). The sera was run in the IEA assay against the vaccine strain and two heterologous strains (1, 4). Strain 161 was not included in the IEA test because it did not adhere to epithelial cells in sufficient numbers (22). The binding (SPRIA) antibody to pili 161 was reduced to below preimmunization levels.

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