Inhibition of Adherence of *Neisseria gonorrhoeae* by Human Genital Secretions

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ABSTRACT Local genital antibodies to the infecting strains of *Neisseria gonorrhoeae* were demonstrated by indirect immunofluorescence (binding antibody) and by their ability to inhibit the attachment of gonococci to epithelial cells (functional antibody). Both IgG and IgA classes of immunoglobulin were involved, and the IgA component were primarily of a secreting (11S) nature. The ability of local genital antibody to inhibit attachment appears to persist for at least a short period of time and to be relatively strain specific.

INTRODUCTION

Infections caused by *Neisseria gonorrhoeae* involve primarily the mucosal surfaces of the cervic, urethra, rectum, and oropharynx and they have been shown to evoke a local antibody response (1, 2). However, the function of these antibodies has remained unclear. Because the initial step in colonization of most pathogens is adherence to the host cell, the ability of these antibodies to inhibit such an occurrence was studied.

This report verifies the stimulation of local antibodies by *N. gonorrhoeae*, documents the ability of those antibodies to inhibit attachment to epithelial cells for at least 3 wk–4 mo, and demonstrates the antibody class that mediates this function.

METHODS

Gonococcal strains. Strains of *N. gonorrhoeae* freshly isolated from patients at the Walter Reed Army Medical Center and strain 9 (kindly supplied by Dr. Douglas Kellogg, Center for Disease Control, Atlanta, Ga.) were used. Bacterial morphology and carbohydrate fermentation reactions were verified periodically. Colonial types (3) were carefully maintained by serial selection. Representative organisms of colonial types 1 and 2 of four strains were shown by electron microscopy to be piliated (4).

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Local secretions. Genital secretions were obtained by instilling 10–15 ml of normal saline with 0.1% NaN_3 into the vagina, taking care to wash the cervix, and then aspirating the specimen. Unless otherwise stated, the specimens were then examined for red blood cells and sperm under light microscopy and discarded if any were present. Male secretions were obtained by collecting from 0.1–0.3 ml of purulent secretion with a tuberculin syringe and suspending it in normal saline. The secretions were sonicated (Branson Instruments, Inc., Stamford, Conn.) for 30 s to break up mucous plugs and then clarified by centrifugation at 141 *g* for 10 min. The supernate was concentrated by filtration (Amicon PM-10 filter, Amicon Corp., Scientific Systems Div., Lexington, Mass.) and stored at −20°C.

Epithelial cell adhesion. Epithelial cell adhesion was modified from the methods of Punsalang and Sawyer (5) and Swanson (6). Gonococci of colony type T1 or T2 were scraped from an 18–20-h culture grown on GC medium (Difco Laboratories, Detroit, Mich.) plus defined supplement (3), suspended in medium 199 (Microbiological Associates, Bethesda, Md.), supplemented with 2% bovine serum albumin, and vortexed to break up large clumps of organisms.

Human buccal epithelial cells, vaginal cells, or cervical cells were scraped with a wooden applicator, suspended in phosphate-buffered saline (PBS), pH 8.0, and washed twice in PBS. The buccal cells were enumerated in a hemocytometer and adjusted in medium 199 to a concentration of 2×10^5 cells/ml. Equal volumes (25 μl) of buccal cells and gonococci (25 μl) were adjusted in medium 199 to a 50:1 ratio of organisms to epithelial cells and incubated at 37°C for 30 min on a shaker apparatus. A slight increase (5–15%) in the number of organisms often occurred in that time interval. Pooled hyperimmune rabbit antisera conjugated with horseradish peroxidase (7) were then used to identify the gonococci by incubating 0.1 ml of the tagged pooled antiserum for 30 min on a shaker apparatus. The cells were washed in normal saline, centrifuged, resuspended in 0.1 ml of normal saline, fixed with 95% ethyl alcohol for 10 min, dried onto slides, and overlaid with 3',3'-diaminobenzidine tetrachloride (Sigma Chemical Co., St. Louis, Mo.) in 0.1 M Tris in 50% ethanol, pH 7.4. The slides were examined under oil immersion and the number of buccal cells with organisms attached was recorded.

Inhibition of epithelial cell adhesion (IEA).[1] IEA was

[1] *Abbreviations used in this paper*: IEA, inhibition of epithelial cell adhesion; IFA, indirect immunofluorescent antibody; PBS, phosphate-buffered saline.

TABLE I

Antibody Concentrations and Indirect Immunofluorescent Antibody (IFA) Titers in Vaginal Secretions

Patient	Date	Immunofluorescent titers*		Immunoglobin		Specific ab titers‡		IgG/IgA
		IgG	IgA	IgG	IgA	IgG	IgA	
				mg/100 ml				
135	9 Jun	<2	<2	2.2	0.4	(0)	(0)	5.4:1
	17 Jun	<2	<2	2.2	0.4	(0)	(0)	5.4
	23 Jun	<2	<2	2.2	0.5	(0)	(0)	4.3
	7 Jul	<2	<2	1.8	0.4	(0)	(0)	4.5
152	7 Jul	32	<2	40.0	4.0	0.80	(0)	10.0:1
	25 Jul	128	<2	42.0	3.9	3.05	(0)	10.8
	8 Aug	16	<2	38.0	3.2	0.42	(0)	11.9
136	17 Jun	8	4	9.8	0.8	0.82	5.00	12.3:1
	22 Jun	32	16	13.5	23.0	2.37	0.70	0.6
	30 Jun	128	<2	3.5	5.9	36.57	(0)	0.6
148	14 Jul	8	64	8.7	4.2	0.92	15.24	2.1:1
	28 Jul	8	8	8.5	3.9	0.94	2.05	2.2
	4 Aug	16	8	17.3	7.8	0.92	1.03	2.2
174	11 Jul	64	64	8.4	1.4	7.62	45.71	6.0:1
	18 Jul	128	64	18.3	5.5	6.99	11.64	3.3
	11 Sep	64	16	9.2	2.7	6.96	5.93	3.4
	19 Sep	32	8	4.3	1.4	7.44	5.61	3.1
134	9 Jun	4	2	33.0	2.2	0.12	0.91	15.0:1
	17 Jun	16	4	8.7	1.7	1.84	2.35	5.1
	23 Jun	32	4	17.0	2.6	1.88	1.54	6.5
	30 Jun	64	16	33.0	13.1	1.94	1.22	2.5
143	7 Jul	128	16	21.1	13.3	6.07	1.20	1.6:1
	14 Jul	64	32	17.0	7.4	3.76	4.32	2.3
	21 Jul	64	8	28.2	12.4	2.27	0.65	2.3
N1	Control	<2	<2	4.2	2.3	(0)	(0)	1.8
N2	Control	<2	<2	3.6	1.9	(0)	(0)	1.9
N3	Control	<2	<2	2.8	1.6	(0)	(0)	1.8
N4	Control	8	<2	7.4	3.0	1.08	(0)	2.5

* Patients vs. homologous strain; control vs. GC9, reciprocal titer.

‡ IFA titer ÷ immunoglobin concentration.

tested by mixing an equal volume of serially diluted local secretion (0.05 ml) to the reactive mixture of epithelial cells and gonococci (0.05 ml). A ≥50% reduction of the number of buccal cells with organisms attached as compared to controls without secretions was considered significant. Other controls included the substitution for the secretion of pooled hyperimmune rabbit antisera made to piliated organisms and epithelial cells without gonococci. In some experiments secretions absorbed with the test strains were also included as a control. Because various gonococcal strains attached to epithelial cells at different percentage rates, the following formula was used to determine the minimum number of epithelial cells to count to assure that a 50% reduction was significant at 95% confidence limits: $n = (2 \times 1.96/d)^2 = 15.37/d^2$ where 1.96 corresponds to an error rate of 5% (using the standard normal distribution) and $d = (2 \text{ arc sin } \sqrt{P_c} - 2 \text{ arc sin } \sqrt{P_{ab}})$ where P_c denotes the "true" fraction of cells in the control with at least one organism attached. P_{ab} denotes the corresponding value in the group receiving antibody with a ≥50% reduction; therefore, $P_{ab} = 0.5 \times P_c$.

The minimum acceptable number of control epithelial cells with organisms attached was 30%. The end pont was usually quite distinct. A 50:1 organism-to-epithelial cell ratio was used to optimize the detection of antibody effect.

Immunofluorescence. Indirect immunofluorescent antibody (IFA) was determined by standard techniques (8). The antigens were gonococcal organisms grown from 18–20 h on GC agar plus defined supplement, suspended in PBS (pH 7.7), washed once, and dried onto slides. Fluorescein-conjugated antisera to human globulins IgG or IgA (heavy chain-specific) (Behring Diagnostics, American Hoechst Corp., Sommer-

ville, N. J., or Hyland Div., Travenal Laboratories, Inc., Costa Mesa, Calif.) or human colostrum absorbed with normal serum (Behring Diagnostics) was used. The human colostrum was conjugated with fluorescein isothiocyanate in our laboratory by to the modified method of Cherry and chromatographed on Sephadex G25 as described by Goldman (9). The fluorescein to protein molar ratios ranged from 2 to 4 and the specific antibody concentration was between 2 and 3 mg/ml.

The slides were incubated at room temperature with human secretions for 20 min, washed in two changes of PBS (10 min each), reincubated with fluorescein-conjugated antisera, rewashed, and mounted in buffered glycerin. Specimens were examined with a Zeiss Universal microscope equipped with a 100× achromatic immersion objective with iris diaphragm, 8× eyepiece and 1.25-Optvar magnifying equipment (Carl Zeiss, Inc., New York). The ultraviolet light source was a 200 W mercury vapor arc lamp. A BG 12 excitation filter and a combination of barrier filters, #53 and #44, were used.

Reactions were recorded as follows: 4+, brilliant fluorescence of all organisms in field 3+, well-defined fluorescence of all organisms in field; 2+, low-intensity but definite fluorescence of 75% or more of the organisms in the field; 1+, occasional organisms with low-intensity fluorescence. A positive reaction was considered 2+ reactivity or greater.

Quantitation of immunoglobulin. Immunoglobulins were quantitated by radial diffusion immunoprecipitation (Mancini method) (10) with commercial low-level plates (Behring Diagnostics). Results were expressed as concentration in milligrams per 100 ml. The reproducibility of the measurements was between 10 and 15%. The quantitative measurements could detect as little as 0.5 mg/ml of the immunoglobulin measured.

Standardization of titers. Specific immunofluorescent titers were standardized on the basis of the concentration in milligrams per 100 ml of secretion by dividing the IFA titer by the quantity of specific immunoglobulin (IgG or IgA). In some experiments the vaginal secretions were adjusted to contain 50% of the 11S IgA standard purified from human parotid saliva (kindly supplied by Jane Suderstrom, Walter Reed Army Institute of Research). This standard equalled 0.19 μg/ml of 11S IgA as determined by computation with an extinction coefficient ($E_{280nm}^{0.1\%}$) of 13.9 (11).

Separation of immunoglobulin. Linear sucrose density gradients (12) were used to separate immunoglobulins in vaginal secretions. Markers included 7S human IgG labeled with ^{125}I (kindly supplied by Wendell Zollinger, Walter Reed Army Institute of Research) and 11S crystalline beet catalase (Worthington Biochemical Corp., Freehold, N. J.) (13). Appropriate fractions were pooled and the dialyzed against 66.7 mM PBS to remove the sucrose, and fianlly against 33.3 mM PBS before lyophilization. Pools were rehydrated to contain no less than 0.10 mg/ml of 11S IgA.

Absorption of secretions. Secretions were absorbed by mixing a heavy suspension of washed organisms, scraped from a 16–18-h-old culture, and incubating the mixture for 1 h at 37°C and then 1 h at 4°C. The organisms were removed by centrifugation. The procedure was performed thrice.

RESULTS

Local antibody response in vaginal secretions. Specific IgA and IgG antibody directed against the homologous (infecting) organism was measured in vaginal secretions by IFA (Table 1). Because the method of collecting the local secretion resulted in variations in the concentrations of immunoglobins, the titers were standardized on the basis of concentration of specific antibody (see Methods). All of the patients were treated when the initial sample was obtained and were cultured negative when follow-up specimens were obtained. Patients 135 and 152 had acute symptomatic gonococcal pelvic inflammatory disease, patients 136, 148, and 174 were asymptomatic gonococcal carriers who probably infected their male partners (i.e. they were named as consorts); and patients 134 and 143 were named as recent contacts (i.e. who infected whom could not be determined).

TABLE II
Immunofluorescent IgA Antibody Titers with 7S and 11S Conjugates

Patient	Date	IgA conjugate 7S	IgA conjugate 11S
134	9 June	2*	2
	17 June	4	2
	23 June	4	4
	30 June	16	16
136	17 June	4	4
	22 June	16	8
	30 June	2	<2

* Reciprocal titers.

Specific IgG could be measured throughout the time interval studied in six of seven cases (Table I). Three patients (152, 136, and 134) demonstrated titer increases: two that peaked (136, 152) and one that plateaued (134). Patients 148 and 174 had levels that remained relatively constant and patient 143 showed a steady decline.

In contrast, the specific IgA antibody level decreased from its peak level in five instances and could not be measured at all in two patients (135 and 152). The two patients (134 and 143) who demonstrated increases in their specific IgA antibody titers were named as recent contacts of infected men and it could not be determined whether they were recently infected or asymptomatic carriers who infected their partners.

Although the IgG/IgA ratios varied for each individual patient, they remained relatively constant after treatment for each individual patient.

Low levels of IgM immunoglobulin could be quantitated in 4 of 10 secretions, but binding to gonococci as determined by IFA could not be detected.

Secretions from four uninfected female patients were also studied. A strain of *N. gonorrhoeae*(GC9) known to cross-react broadly in immunofluorescent studies was used as the antigen (12). None of the vaginal secretions had detectable specific IgA antibody, while one patient who was menstruating demon-

TABLE III
Local Antibodies to N. gonorrhoeae *in Male Urethral Secretions*

Patient	IFA titer*		Immunoglobulin		Specific titer‡		
	IgG	IgA	IgG	IgA	IgG	IgA	IgG/IgA
			mg/100 ml				
7051	32	2	14.2	4.0	2.25	(0)	3.6
7105	32	4	10.2	1.3	3.14	3.08	7.9
7102	4	<2	1.1	0.6	3.64	(0)	1.8
7107	8	8	6.2	1.8	1.29	4.44	3.4

* Reciprocal titer vs. GC9, indirect immunofluorescence.
‡ IFA titer ÷ immunoglobulin concentration.

strated specific binding of IgG. The IgG/IgA ratio in these patients was remarkably consistent (Table I).

Secretions from two patients, 134 and 136, were also studied with fluorescein-tagged antihuman colostrum antibody absorbed with normal human serum to render it 11S-specific (Table II). The local IgA response was principally of an 11S nature (secretory IgA antibody).

Urethral secretions from four male patients with acute gonococcal urethritis were studied by immunofluorescence with GC9 as the antigen (Table III). The antibody response was similar to that in the females. Sequential samples could not be obtained since significant urethral discharge ceased with appropriate therapy.

IEA. Vaginal secretions from infected patients could inhibit epithelial cell adhesion of the homologous strain in four out of six instances (Table IV). This activity was relatively specific, i.e. epithelial cell adhesion of the infecting organism was blocked at a higher titer than a heterologous organism. Secretions from uninfected females could not block epithelial cell adhesion of the four strains tested. Both of the patients (135 and 152) who could not inhibit their infecting strain had no IgA detectable by IFA, and patient 135 also lacked specific IgG antibody. No quantitative differences in the ability of gonococci to attach to buccal, cervical, or vaginal cells were demonstrated in three simultaneously determined instances.

Urethral secretions from four males with acute gonococcal urethritis were also capable of inhibiting epithelial cell attachment of their infecting organisms.

Vaginal secretions were collected over a 4-mo period from a single patient (Table V). In this study all of the secretions were standardized to 50% of an 11S IgA standard (see Methods). The patient had had contact with an infected male 28 days before the initial specimen was obtained and was admitted to the hospital with a disseminated gonococcal infection. Her IEA level remained constant over that period of time, but the relative amount of specific antibody attributable to specific IgA or IgG antibody shifted (Table V).

To determine which antibody class was responsible for the IEA, secretions from Jan. 29, Feb. 9, and Mar. 19 were fractionated in linear sucrose density gradients (Fig. 1). Appropriate fractions were pooled, dialyzed, lyophilized, reconstituted, and tested. Both 11S (IgA) and 7S (IgG) antibodies were responsible for blocking epithelial cell attachment, but the proportion of the antibody class involved shifted over the 4 mo (Table VI). No IgM antibody was detected in the 11S pool.

Although immunofluorescent antibody (IgG) could be measured in only two of five vaginal secretions from uninfected females, all five uninfected patients were capable of inhibiting attachment of strain 418

TABLE IV
IEA by Vaginal Secretions

Patient	Date	IEA		Specific Ab titers, Immunofluorescence	
		Homologous*	Heterologous‡	IgG	IgA
134	9 Jun	64	8,8,8	0.12	0.91
	17 Jun	256		1.84	2.35
	23 Jun	128	8,8,8	1.88	1.54
	30 Jun	8		1.94	1.22
135	9 Jun	<4		(0)	(0)
	17 Jun	<4		(0)	(0)
	23 Jun	<4		(0)	(0)
	7 Jul	<4		(0)	(0)
136	17 Jun	128	<4	0.82	5.00
	22 Jun	128	<4	2.37	0.70
	30 Jun	128	<4	36.57	0.00
143	7 Jul	8		6.07	1.20
	14 Jul	8		3.76	4.32
	21 Jul	<8		2.27	0.65
152	1 Aug	<4		0.80	(0)
	8 Aug	<4		3.05	(0)
	21 Aug	<4		0.42	(0)
149	7 Jul	8		ND	
	25 Jul	8		ND	
	8 Aug	8		ND	
N1			<4§ <4<4<4		
N2			<4 <4<4<4		
N3			<4 <4<4<4		
N4			<4 <4<4<4		

* IEA vs. infecting strain.
‡ IEA vs. heterologous strain.
§ IEA vs. one of the above infecting strains.

(Table V) at a low titer. No IgA antibody was detected by immunofluorescence.

To determine whether indirect IFA measured the same antigen(s) involved in attachment of the gonococci to epithelial cells, genital secretions from two patients were absorbed with nonpiliated colony type 3 or type 4 homologous organisms. Although the titers decreased two- to fourfold, IFA and IEA antibody persisted, suggesting that the antigen(s) responsible for attachment (and therefore inhibited) was measured by both assays. Absorption of the same secretions with piliated type 1 or type 2 homologous organisms reduced the IFA and IEA titers to base-line levels.

DISCUSSION

Kearns et al. (1) and O'Reilly et al. (2), using similar immunofluorescent techniques, demonstrated the presence of local antigonococcal antibody in patients infected with *N. gonorrhoeae*. Their studies concentrated on demonstrating the presence of secretory IgA and quantitating its level over a period ranging from a few days to 9 mo. They used a single, broadly cross-reactive strain (GC9) as the antigen (14). In the present study the homologous infecting strain was used as the antigen and the patients were followed for a shorter period of time. Nevertheless, the findings were quite comparable.

TABLE V
Inhibition of Epithelial Cell Attachment of Homologous Strain over 4 mo

Date	IEA GC418	IgG*	IFA IgA	IFA IgG	Specific IgG
		mg/100 ml			
29 Jan	64‡	5.6	16‡	4‡	0.71
31 Jan	64				
6 Feb	16	4.4			
8 Feb	32	4.4			
19 Feb	32	4.8	8	32	6.67
4 Mar	64	15.0	8	64	4.27
23 Apr	32	10.4	8	64	6.15
19 May	64	5.5	4	32	5.82
Uninfected secretions					
N 9	2	>12.5	<2	<2	0.00
N 12	8	1.2	<2	2	1.67
N 14	4	1.6	<2	<2	0.00
N 15	4	7.4	<2	4	0.54
N 16	2	2.2	<2	<2	0.00

* Secretions were standardized to 50% of an 11S standard; therefore, IgA titers can be compared directly and need not be standardized (See Methods).
‡ Reciprocal of titer

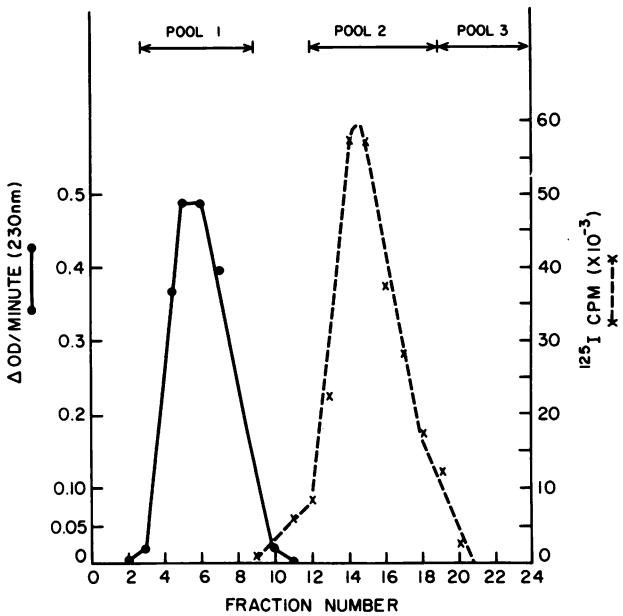

FIGURE 1 Fractionation of vaginal secretions on sucrose density gradient. The 7S marker was purified IgG iodinated with I^{125}; the 11S marker was beef crystalline catalase. Markers and secretions were run on different gradients at the same time. Fractions from the secretion gradients corresponding to the marker peaks were pooled and tested.

The present studies confirm the earlier findings that the local IgA antibody response was principally secretory (11S). Of the six female patients O'Reilly et al. (2) studied sequentially, two had a rapid fall in specific IgA antibody titer (≤10 days), three had decreased to base-line levels by 50 days, and one patient had persistent high levels for more than 150 days. One of five patients with specific IgA antibody in this study had a rapid decrease to undetectable levels after treatment, while the remaining four patients with specific IgA had persistent but declining levels of IgA antibody at 3–4 wk.

Specific IgG antibody binding to the homologous infecting strains was also demonstrated. These antibodies were present in 10 of 11 patients and persisted throughout the time interval studied. The single patient (135) without specific IgG antibody had detectable IgG by radial-immunodiffusion and IFA when GC9 was used as the antigen, suggesting that she lacked specific IgG directed against strain 135.

Two patients, both of whom had symptomatic acute pelvic inflammatory disease, had no specific IgA antibody, although patient 135 did have IgA antibody to strain GC9, again suggesting a lack of strain-specific response to GC135. Patient 152 did mount a detectable strain-specific IgG response.

The three patients (136, 148, 174) who were asymptomatic carriers had the highest initial specific

TABLE VI
The IEA of IgA and IgG Fractions in Vaginal Secretions

Date	Unfractionated secretions*	Fractions		
		11S (pool 1) IgA	7S (pool 2) IgG	Pool 3‡
29 Jan	64§	8	2	<2
9 Feb	64	4	2	ND
19 May	64	4	16	ND

* Unfractionated vaginal secretions tested the same day.
‡ Pool 3 tested as a control.
§ Reciprocal titers.

IgA titers, presumably because of prolonged stimulation by the infecting strains. In each instance the IgA level decreased precipitously while the IgG level remained constant in two and increased in the other. Both patients (134 and 143) named as recent contacts, i.e. cultured as part of epidemiologic follow-up, demonstrated specific IgA titer increases that might represent a response to recent acquisition of gonococci.

The IgG responses did not appear to fall into any pattern that corresponded with the clinical status of the patient as closely as the IgA responses. This may be due to patient variations in the amount of transudation of IgG from serum with or without concomitant local production (preliminary studies revealed IEA activity in serum also). Most of the secretory IgA, on the other hand, is probably made locally (11, 15) and may reflect more closely the immunological events taking place on the local surface.

The concentrations of immunoglobulins varied as a result of the dilution of the specimen during collection. Nevertheless, the IgG/IgA ratios were consistent with previous studies that demonstrated a wider scatter of immunoglobulin levels in infected patients (16). Three patients (134, 136, 174) had their highest concentration of IgG at the time of active infection, which then decreased after treatment. Presumably this is a reflection of the inflammation on the local mucosal surface before treatment. Four patients (135, 143, 148, and 152) maintained relatively constant ratios. However, no consistent clinical presentation existed in these two groups. The ratios reflected relatively lower concentrations of IgA as a result of the 7S IgA standard. For example, Waldman et al. (17) determined specific 11S IgA antibody and reported IgG/11S IgA ratios between 1:2 and 1:4, which would mean that the secretory IgA concentrations may be as much as 10 times higher than reflected in this study.

How local antibody might protect the host is unclear. Recently, the prevention of attachment by local antibodies of an organism to mucosal cells has been demonstrated. Williams and Gibbons (18) have demonstrated the capacity of secretory IgA to inhibit adherence of cariogenic organisms to human buccal cells. Steele et al. have also demonstrated the capacity of all three classes of immunoglobulins given orally to protect infant mice from *Vibrio cholerae*, presumably by inhibiting adherence to intestinal mucosal cells (19). Studies with rabbit antisera have been successful in blocking epithelial attachment of *N. gonorrhoeae* to a number of different cells (5, 20–22). The present studies demonstrate that human local secretions have the same ability, and that this capacity appears to be immunologically mediated through both IgG and secretory IgA antibodies. In the single patient studied over a 4-mo period, the IEA titer was maintained between 1:16 and 1:64. At the onset this function was due primarily to 11S IgA, but shifted to IgG before the patient was lost to follow-up. Whether this is because she had had a disseminated infection and, therefore, substantial antigenic stimulation resulting in subsequent prolonged transudation of specific IgG into the vagina, or whether the specific IgG was made locally and represents a common sequence of events is not known. Patient 136, an asymptomatic carrier, also demonstrated an apparent similar shift (Tables I and IV). On 30 Jun she had no specific IgA antibody as demonstrated by immunofluorescence, but nevertheless was able to block epithelial cell attachment, presumably mediated by IgG.

All five vaginal secretions from uninfected patients inhibited epithelial cell attachment of strain 418, although only low titers of IgG could be measured by IFA in only two of them. However, two points must be kept in mind: (*a*) indirect IFA, which measures antibody that binds to the whole organism dried and fixed onto a slide, and IEA are antibody determinations that most likely detect (share) only a few common antigens, and (*b*) the relative sensitivity of each test cannot be estimated without knowledge of which specific antigens are involved. However, when the genital secretions were absorbed with nonpiliated organisms that lack those antigens mediating attachment (6, 23–25), immunofluorescent antibody persisted. Further absorption with piliated organisms reduced the IFA titer to base-line levels. Thus, it would appear that both antibody tests were measuring the same antigens and that IEA is a more sensitive measurement than IFA of the antibody mediating epithelial cell attachment.

Both patients incapable of blocking epithelial cell adhesion (135 and 152) had symptomatic acute pelvic inflammatory disease. Although the content of immunoglobulin IgG and IgA could be quantitated, neither patient had specific immunofluorescent IgA antibody, while one (135) also lacked specific IgG antibody. One could speculate that (*a*) neither patient had time to mount a local antibody response because they sought treatment early or (*b*) they developed an acute symptomatic illness because of an inability to

block epithelial cell adhesion and, therefore, resist colonization and subsequent tissue invasion. Indeed, all vaginal secretions recovered from asymptomatic patients (carriers) developed IEA antibody.

Virulence of gonococci has been shown to correlate with colonial types 1 and 2 (3, 26). Numerous studies have documented the increased ability of these colonial types to adhere to mammalian cells (6, 23–25). With rabbit antisera, inhibition of adherence appears to be relatively specific (22, 27–29), and an analogous situation appears to be true for vaginal secretions. In contrast to the homologous strain, patient 134 was able to block three heterologous strains at a low titer that did not change over 12 days (Table IV), while patients 136 (Table IV) and 418 (unpublished observations) were unable to inhibit attachment of heterologous organisms at 1:4 and 1:2 dilutions, respectively. Also, secretions from four uninfected patients were incapable of inhibiting attachment of four different strains (Table IV). In contrast, all five uninfected patients demonstrated some low level blocking activity against strain 418, suggesting that a significant amount of "natural" antibody directed against this strain existed. It is interesting that patient 418 was unable to infect any of her subsequent male partners (at least two, one of with whom she had at least eight contacts). Also, strain specificity of IEA may help explain the recurrent nature of gonococcal infections.

Recently, gonococci have been shown to elaborate extracellular proteases that can cleave IgA into Fab and Fc fragments (30). However, whether this would alter IEA activity must remain to be seen, since if all that is required for this effect is binding of the Fab fragment to the pertinent antigen (31), then its ability to block attachment may be unimpeded (32).

The nature of gonococcal disease makes it very difficult to be sure with any degree of certainty when the infection actually began. For example, the incubation period as well as the infectivity rate (33) is quite variable, and the patient often has had multiple exposures to one or more partners. Thus, only the dates when specimens were obtained were listed.

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