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Specificity of Inhibition of Epithelial Cell Adhesion of *Neisseria gonorrhoeae*

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Inhibition of epithelial cell adhesion (attachment) for individual strains of *Neisseria gonorrhoeae* is antigenically distinct.

Colonies of *Neisseria gonorrhoeae* can be classified as various colony types, but only colony types 1 and 2 are generally considered pathogenic for man (7). A distinguishing characteristic of these colonial type organisms is the presence of pili (14). Buchanan, using antigen-antibody binding techniques (immunofluorescent antibody [5] and radiolabeled antibody [4]), found that a slight degree of cross-reactivity existed between pili isolated from different strains, but that each of two purified pili preparations inhibited antibody binding to the homologous preparation only (3) and that hemagglutination was inhibited by the homologous antisera only of two additional strains (total four strains) (4). Novotny and Turner (9) demonstrated a similar degree of specificity. Pili are considered mediators of attachment (adhesion) to various mammalian cells (6, 10–12, 14, 15), and antisera made to pili have been shown to block attachment of gonococci to these cells (8, 10, 12). By measuring inhibition of epithelial cell attachment, the present study verifies that the antigenic determinants of attachment, presumably pili, vary for each individual strain.

Gonococci of colony type T1 or T2 were scraped from an 18- to 20-h culture grown on GC medium (Difco Laboratories, Detroit, Mich.) plus defined supplement (7), suspended in medium 199 (Microbiological Associates, Bethesda, Md.) supplemented with 2% bovine serum albumin, and vortexed to break up large clumps of organisms.

Antisera made in rabbits to piliated organisms were absorbed with nonpiliated organisms of the same strain to render them specific for pili as previously described (16) (i.e., antisera would bind only to piliated colonies as determined by immunofluorescence and inhibited hemagglutination of rabbit erythrocytes).

Epithelial adhesion was determined as follows. Human buccal epithelial cells were scraped from noninfected volunteers with a wooden applicator, suspended in phosphate-buffered saline (PBS), pH 8.0, and washed two times. The buccal cells were enumerated in a hemocytometer and adjusted in medium 199 (Microbiological Associates) to a concentration of 2×10^5 cells/ml. Equal volumes (0.025 ml) of buccal cells and gonococci (0.025 ml) were adjusted in medium 199 to be at a 500:1 ratio of organisms-epithelial cell and incubated at 37°C for 30 min on a shaker apparatus. A slight increase (5 to 15%) in the number of organisms often occurred in that time interval. Pooled hyperimmune rabbit antisera (16) conjugated with horseradish peroxidase (1) were then used to identify the gonococci by incubating 0.1 ml of the pooled antiserum for 30 min on a shaker apparatus. The cells were washed in normal saline, centrifuged, resuspended in 0.1 ml of 0.85% NaCl, 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(hydroxymethyl)aminomethane 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 adhesion was carried out by mixing an equal volume of antiserum (0.05 ml) and the reaction mixture of buccal cells and gonococci. A $\geq$50% reduction was considered significant. Controls included antisera made to colony type T3 organisms, preimmunization antisera, and buffer (PBS, pH 8.0) substituted for the antisera.

Antisera inhibited attachment of the homologous organism at the highest titer in every instance, although broad cross-reactivity at low titers was evident (Table 1). Preimmunization antisera, or antisera made to nonpiliated organisms, did not inhibit attachment.

Crude pili preparations were made from two strains (104, 125) by a modification of a method by Brinton (2). Briefly, colony type 2 gonococci

TABLE 1. *Inhibition of epithelial cell adhesion by rabbit antisera*

Rabbit antisera[a]	Organisms											
	9	101	103	104	105	108	110	113	120	121	125	129
9	16[b]	2	2	2	<2	<2	2	4	<2	<2	2	<2
101	<4	512	4	16	2	4	<2	<4	64	2	16	<2
103	4	2	512	2	2	2	2	2	<2	2	4	<2
104	<4	<4	4	64	2	<4	<2	2	<4	<2	<4	<2
105	2	2	2	2	128	4	2	4	<2	4	2	<2
108	4	8	2	4	<2	32	2	<2	4	<2	2	<2
110	2	2	4	2	<2	2	64	2	<2	2	<2	<2
113	2	2	<2	<2	2	2	<2	32	2	2	2	<2
120	ND[c]	4	<2	8	4	2	<2	2	32	2	8	4
121	ND	2	4	<2	2	2	32	4	<2	512	4	<2
125	ND	4	2	8	2	8	<2	2	<4	4	1,024	2
129	ND	2	2	<2	2	<2	<2	<2	<2	<2	<2	256

[a] Antisera to piliated organisms absorbed with unpiliated organisms.
[b] All numbers are reciprocal titers.
[c] ND, Not done.

were scraped from an 18- to 20-h culture and suspended in PBS, pH 8.0 ± 0.01, and blended at top speed in an Omnimixer (Sorvall, Norwalk, Conn.) for 2 min at 4°C. The organisms were removed by centrifugation, and the supernatant was adjusted to pH 6.5 with 1 N HCl and held overnight at 4°C. The aggregate was collected by centrifugation and dispersed in PBS, pH 7.2 ± 0.01. After addition of $MgCl_2$ to a final concentration of 0.1 M, the aggregates were collected by centrifugation. The presence of pilus antigen was demonstrated by electron microscopy. Antisera made to these preparations gave identical results. Also, mixing and incubating these preparations with the homologous antisera made to the piliated organisms at 37°C for 30 min before diluting the antisera blocked the inhibitory effect of these antisera.

Two gonococcal strains isolated from a pair of consorts and shown to be identical (E. C. Tramont, J. M. Griffiss, D. L. Rose, G. F. Brooks, and M. S. Artenstein, J. Infect. Dis., in press) were blocked by antisera made to each of these strains.

Although these results are indirect, they suggest that the antigen(s) of *N. gonorrhoeae* responsible for attachment to buccal cells is quite antigenically distinct for each individual organism. Presumably, this antigen(s) is pilus, although another antigen(s) cannot be ruled out. Nevertheless, these cross-reactive but distinguishable antigens may be useful as an epidemiological tool for examining relatedness of individual strains. Also, if the human immune response to the gonococcal pilus antigen represents the main antigenic thrust of that response, then this relatively narrow specificity would render antibody to pili unlikely to be protective against a large number of organisms, and frequent reinfection with other strains would be anticipated, a common occurrence in gonococcal infections.

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