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Stimulation of Human Leukocytes by Protein II$^+$ Gonococci Is Mediated by Lectin-Like Gonococcal Components

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We investigated the ability of carbohydrates, glycosidases, and concanavalin A to inhibit the stimulation of the human leukocyte oxidative burst by gonococci in the absence of serum. The gonococci used in this study contained protein II (P.II) outer membrane proteins, and neutrophil oxidative burst was measured by luminol-enhanced chemiluminescence (CL). The following carbohydrates inhibited CL induced by nonpiliated P.II$^+$ gonococci: beta-D-glucosamine > *N*-acetylneuraminic acid (sialic acid) > mannose > alpha-methylmannoside > *N*-acetyl-beta-D-glucosamine ≥ glucose ≥ lactose. Fucose, galactose, or beta-D-galactosamine (all 100 mM) did not inhibit or slightly increased CL, indicating a specificity for the observed effects. Mannose and alpha-methylmannoside also inhibited induction of monocyte CL by P.II$^+$ gonococci. Incubation of neutrophils with concanavalin A inhibited subsequent gonococcus-induced CL but not phorbol myristate acetate-induced CL. Treatment of neutrophils with alpha-mannosidase reduced subsequent gonococcus-induced CL >99%, whereas such treatment of gonococci had no effect on their ability to induce neutrophil CL. Incubation of a P.IIb-containing variant of *Neisseria gonorrhoeae* FA1090 with anti-P.IIb monoclonal antibody inhibited subsequent stimulation of neutrophil CL in a dose-responsive manner, indicating a specific role for P.IIb in the stimulatory process. The data suggest that one or more lectin-like components on the surface of P.II$^+$ gonococci mediate their ability to stimulate the oxidative burst of human phagocytes.

During both localized and disseminated gonococcal infections neutrophils, monocytes, and macrophages interact intimately with gonococci in blood and tissue (16, 28). Available evidence suggests that most gonococci, once phagocytosed, are efficiently killed by human neutrophils and monocytes (8, 14, 26, 38, 53, 54). This killing can be attributed either to leukocyte oxidative mechanisms, because gonococci stimulate human leukocytes to undergo an oxidative burst (8, 38), or to nonoxidative mechanisms, because gonococci are readily killed by neutrophils from patients with chronic granulomatous disease (38). Although pili seem to protect gonococci from being ingested and killed by human phagocytes (29, 36, 53, 54), there has been serious question raised by Swanson and his colleagues whether other gonococcal outer membrane components, especially leukocyte association proteins, or P.IIs, might actually be more important mediators of gonococcus-neutrophil interactions (21, 50–52); P.IIs might counter the antiphagocytic effects of pili. In this study, we continue our investigations into the role of gonococcal outer membrane components in the interaction of P.II$^+$ gonococci with human phagocytic cells. Gonococci possessing one or more P.IIs have been shown to adhere to several different cell types (13, 25, 31, 44, 57, 58), to be phagocytosed in the absence of serum by human neutrophils and monocytes (26, 38, 40) and by *Trichomonas vaginalis* (11), and to induce human neutrophil and monocyte chemiluminescence (CL) (38) to a greater extent than gonococci lacking P.II. In addition, P.II$^+$ gonococci appear to be more sensitive to serum bactericidal activity (17) than are P.II$^-$ gonococci. P.IIs thus appear to be major factors in gonococcal virulence.

Although various proteases, glycosidases, and other treatments modulate the adherence of nonpiliated P.II$^+$ gonococci to host cells (20, 44, 58), no clear hypothesis has yet evolved as to the molecular mechanisms involved in such interactions. Several gram-negative bacteria adhere to host cells by mechanisms involving lectin-like components specific for a number of simple monosaccharides and amino sugars (2, 10, 30, 35, 41–43, 45, 58). Little information exists, however, on mechanisms of bacteria-host cell interactions (mediated by lectin-like components) that perhaps are more subtle than adherence. Although lectins were initially defined as plant seed carbohydrate-binding proteins, their occurrence in bacteria and many other organisms has led to an expansion of this definition. Goldstein et al. have more recently suggested that lectins possess at least the following characteristics. They are soluble or membrane bound; possess the ability to precipitate glycoconjugates or agglutinate plant or animal cells (i.e., they have at least two carbohydrate-binding sites); and are defined in terms of the monosaccharide(s) or simple oligosaccharide(s) that inhibit lectin-induced glycoprecipitation or agglutination (15). If one looks at the stimulation of human neutrophils by lectins such as concanavalin A (ConA) or wheat germ agglutinin, one can see a working model of this definition as it is applied to precipitation or agglutination of glycoconjugates (receptors) within the plane of the membrane of a eucaryotic cell (6, 7, 32, 34, 56). Such mechanisms might be involved in neutrophil stimulation by bacteria, such as that described in the present report, or in inhibition of phagocyte oxidative responses by pathogens, such as that which has been observed for *Salmonella typhi* (27), *Brucella abortus* (23), and *Toxoplasma gondii* (59), among others.

MATERIALS AND METHODS

Gonococci. *Neisseria gonorrhoeae* strains were maintained by daily transfer on GC medium base (Difco Laboratories, Detroit, Mich.) with supplements, as described previously

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(37, 39). Colony types were identified by the criteria described by Kellogg et al. (18, 19) and Swanson (47, 48). Unless otherwise stated, experiments were performed with strain F62. For daily use, gonococci were grown to mid-log phase in GC broth with supplements, washed once (12,000 × *g*, 1.5 min, room temperature), and suspended in Hanks balanced salt solution containing 0.1% gelatin (pH 7.4; HBSG) at 10^7 to 10^8 CFU/ml, as described previously (37, 39). To confirm that our observations were not limited to strain F62, several experiments involving mannose were repeated with P.II$^+$ variants of GC7, a serum-sensitive strain described previously (9, 39), and with FA1090, a serum-resistant strain kindly supplied by Janne Cannon of the University of North Carolina, Chapel Hill; results (not presented) were similar for all three strains. All the strains used in this study yield variants possessing specific P.IIs which are identifiable by polyacrylamide gel electrophoresis. We regularly use polyacrylamide gel electrophoresis to assure ourselves that we are indeed working with the proper variants. In this study, strain F62 had two P.IIs, as described previously (39); strain GC7 had a single P.II, as described previously (9, 39); and strain FA1090 contained P.IIb, as recently described by Black et al. (3) and Sugasawara et al. (44).

Leukocytes. Human neutrophils and monocytes were purified from freshly obtained heparinized venous blood by a single centrifugation through Ficoll-Hypaque, as described by Ferrante and Thong (12). Erythrocytes were removed from neutrophil suspensions with one hypotonic lysis with distilled water. Neutrophils were >94% pure as determined by Wright stain and ≥98% viable as determined by exclusion of 0.25% Trypan blue in phosphate-buffered saline. Mononuclear cell suspensions contained 57% lymphocytes, 37% monocytes, 4% basophils, and 2% neutrophils, with essentially no erythrocyte contamination, and were >97% viable, as described previously (26). Leukocytes were washed once (150 × *g*, 8 min, 3°C) and suspended in HBSG to 1 × 10^7 to 5 × 10^7/ml and kept on ice until use.

CL. Luminol-enhanced CL was measured in polyethylene scintillation vials containing 10^6 leukocytes, 1 × 10^7 to 5 × 10^7 gonococci, 10^{-5} M luminol (added as 10^{-3} M luminol in dimethyl sulfoxide), appropriate additions, and HBSG to 1 ml, as described previously (38, 55). Vials were counted in various scintillation counters set in the in-coincidence mode with tritium preset windows at approximately 28°C for 6 s each and recounted at regular intervals (between 5 and 10 min) with no additional mixing. Serum was not present in the assays. Results are expressed as percentage of control ± standard deviation (SD) and were computed as [(peak CL response of experimental samples)/(peak CL response of control samples)] × 100. When the number of experiments was only 1 or 2, i.e., when $n \leq 2$, results are presented with no mean. Peak CL values in untreated controls (containing neutrophils, luminol, and gonococci in a final volume of 1 ml of HBSG) ranged from 100,000 to 800,000 cpm on different days.

Superoxide production. A continuous assay of superoxide dismutase-inhibitable cytochrome *c* reduction was used to measure superoxide production, as described by Babior and Cohen (1). A Perkin-Elmer Lambda 3B dual-beam spectrophotometer was used. Results are reported as percentage of control and were computed as [(maximal change in absorbance per minute in the presence of mannose)/(maximal change in absorbance per minute in the absence of mannose)] × 100.

Oxygen consumption. Oxygen consumption was measured by using Clark oxygen electrodes and a model 53 oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio) attached to a Perkin-Elmer model R100 recorder by methods described by Babior and Cohen (1) with slight modifications. Reaction mixtures containing 2 × 10^7 neutrophils and appropriate additions were brought to a final volume of 3 ml with HBSG. Neutrophils were equilibrated at 37°C for 10 min in HBSG in the water-jacketed oxygen monitor reaction vessels before the addition of appropriately treated gonococci.

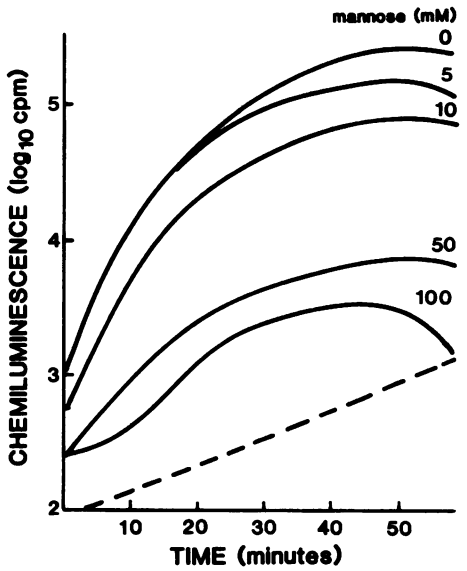

FIG. 1. Representative CL assay. Reaction vials contained 10^6 neutrophils, 3 × 10^7 gonococci (strain F62), 10^{-5} M luminol, and mannose as indicated in a final volume of 1 ml of HBSG. P.II$^+$ (solid lines) or P.II$^-$ (broken line) gonococci were incubated 15 min with mannose or HBSG before the addition of neutrophils to initiate CL.

Reagents. Carbohydrates, neuraminidase (EC 3.2.1.18, type V, from *Clostridium perfringens*), Ficoll (type 400), ConA and succinylated ConA (suc-ConA) were purchased from Sigma Chemical Co., St. Louis, Mo. All carbohydrate solutions were 1 M in double-deionized water (except lactose, which was 0.5 M) and were adjusted to pH 7 with 10 N KOH. Stock carbohydrate solutions were stored at −20°C. Hypaque-M, 75% was purchased from Winthrop Laboratories, Div. Sterling Drug Inc., New York, N.Y. Alpha-D-mannosidase (EC 3.2.1.24; from jack bean) was purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. The murine monoclonal antibody to P.IIb from gonococcal strain FA1090 was kindly supplied as ascites fluid by Janne Cannon. The specificity and several biological activities of this monoclonal antibody were recently described (3, 44). Murine ascites fluid containing MOPC-21 [immunoglobulin Gl(κ)], induced by BALB/c myeloma P3X63-Ag8, was kindly supplied by Carole Long, Hahnemann University, and was used as a nonspecific monoclonal antibody.

RESULTS

Gonococci alone or in the presence of carbohydrates produced no CL above background (≤100 cpm), and neutrophils alone or in the presence of carbohydrates occasionally produced up to 0.25% of peak CL (<1,000 cpm). Gonococcus-induced neutrophil CL increased steadily until peak CL was reached and then decreased, as shown by a representative experiment (Fig. 1). P.II$^-$ gonococci induced only minimal CL (38; Fig. 1). Unless otherwise indicated,

TABLE 1. Effects of carbohydrates on neutrophil CL induced by *N. gonorrhoeae*

Carbohydrate and amt (mM)[a]	CL induction	
	No. of expts	% of control[b]
Mannose		
100	7	3.0 ± 2.0
50	4	8.6 ± 7.0
10	5	38.8 ± 17.5
5	4	52.1 ± 11.5
Alpha-methylmannoside		
100	6	24.2 ± 17.4
50	3	53.7 ± 15.7
10	3	85.2 ± 13.4
5	3	92.9 ± 9.0
Beta-D-glucosamine		
100	4	0.09 ± 0.08
30	4	15.0 ± 11.8
10	4	82.7 ± 42.1
3	4	123.0 ± 60.1
N-acetyl-beta-D-glucosamine		
100	4	41.0 ± 4.0
30	2	74.9–86.2
10	2	78.2–87.6
3	2	100.8–116.8
Sialic acid		
100	3	0.8 ± 0.35
50	3	10.1 ± 2.1
25	3	24.7 ± 10.0
10	3	51.2 ± 4.2
5	2	51.1–80.0
Glucose (100)	4	43.4 ± 2.6
Lactose (50)	4	65.8 ± 5.4
Fucose (100)	4	180.2 ± 60.7
Galactose (100)	4	191.7 ± 90.9
Beta-D-galactosamine (100)	3	169.7 ± 85.2

[a] Gonococci were incubated 15 min at 37°C with carbohydrate before the addition of untreated neutrophils.

[b] Controls contained no carbohydrate and had peak CL values ranging from 100,000 to 800,000 cpm on different days. Data are expressed as mean ± SD when $n > 2$.

the mediators discussed below only slightly altered the time to reach peak CL (a range of 39 to 58 min was obtained for most experiments), regardless of their effects on the magnitude of peak CL. To indicate that mannose indeed inhibited gonococcus-induced neutrophil oxidative burst, not simply CL, we investigated the effects of 100 mM mannose on superoxide production and oxygen consumption by gonococcus-stimulated neutrophils. Mannose, when preincubated with gonococci for 15 min at 37°C or when added simultaneously with gonococci, inhibited gonococcus-induced neutrophil superoxide production by 78.5 ± 13.5% ($n = 3$) and oxygen consumption by 57.1% ($n = 2$; range, 51.0 to 63.3%). Superoxide production and oxygen consumption were thus also inhibited by 100 mM mannose.

Modulation of gonococcus-induced leukocyte CL by carbohydrates. To investigate the possible involvement of lectin-like components in gonococcal stimulation of human phagocytic cells, gonococci were incubated with one of several carbohydrates in HBSG for 15 min at 37°C before being added to neutrophils to initiate CL. The following carbohydrates (at concentrations of 100 mM) inhibited CL, in order of decreasing efficacy: beta-D-glucosamine > *N*-acetylneuraminic acid (sialic acid) > mannose > alpha-methylmannoside > *N*-acetyl-beta-D-glucosamine ≥ glucose ≥ lactose. All these carbohydrates inhibited gonococcus-induced neutrophil CL in a dose-dependent manner (Table 1). Mannose (100 mM) and alpha-methylmannoside (200 mM) also inhibited gonococcus-induced monocyte CL to 3.5 ± 1.6% ($n = 3$) and 52.9 ± 7.9% ($n = 3$) of controls, respectively (data not shown). Preincubation of gonococci with 100 mM fucose, galactose, or beta-D-galactosamine led to a slight increase in CL induced by untreated gonococci (Table 1). Neutrophils remained ≥95% viable throughout the experimental procedures, as measured by Trypan blue exclusion, indicating that the observed decreases in CL were not due to neutrophil death.

The different efficacies with which the various carbohydrates inhibited CL and the complete lack of inhibition of CL by 100 mM fucose, galactose, or galactosamine indicated that the inhibitory effects observed were not due to nonspecific effects of high carbohydrate concentrations; however, to specifically investigate this question and to see which of the two cells (the gonococcus or the neutrophil) possessed relevant mannose-binding component(s), the following experiments were done. Gonococci were preincubated with 100 mM mannose and then washed once (12,000 × *g*, 1.5 min, room temperature) before use in CL assays. Gonococci pretreated in this fashion induced 26.2 ± 9.9% ($n = 4$) of the amount of CL induced by untreated gonococci. Neutrophils exposed to 100 mM mannose for 15 min at 37°C, washed (150 × *g*, 8 min, 3°C), and incubated with untreated gonococci showed no decreased CL compared with washed neutrophils not exposed to mannose (washed control peak CL = 740,597 ± 22,747 cpm; washed mannose-treated peak CL = 744,940 ± 50,611 cpm; $n = 3$). These data indicate the presence of a mannose-containing receptor on the neutrophil and a mannose-specific ligand on the gonococcus.

Effects of glycosidases on gonococcus-induced neutrophil CL. To better establish the presence of a mannose- or sialic acid-containing receptor on the neutrophil surface, neutrophils were preincubated with various concentrations of alpha-mannosidase or sialidase for 15 min at 37°C before the addition of untreated gonococci. Alpha-mannosidase inhibited subsequent gonococcus-induced CL by >99%, whereas sialidase treatment slightly stimulated subsequent CL (Table 2). Gonococci pretreated with 100 U of alpha-mannosidase per ml (the highest concentration of enzyme used in these assays) for 15 min at 37°C and then washed (12,000 × *g*, 1.5 min, room temperature) stimulated CL to the same extent as did untreated gonococci (data not shown).

TABLE 2. Effects of glycosidases on neutrophil CL induced by *N. gonorrhoeae*

Glycosidase and amt (U/ml)[a]	CL induction (mean % ± SD of control [no. of expts]) vs. control stimulated with:	
	Gonococci	PMA
Alpha-mannosidase		
100	0.35 ± 0.05 (3)	44.4 ± 3.1 (6)
30	12.1 ± 13.6 (4)	59.5 ± 3.4 (3)
10	41.3 ± 8.1 (4)	89.3 ± 4.1 (3)
3	70.0 ± 16.8 (4)	
Sialidase (0.1)	124.0 ± 27.3 (4)	

[a] Neutrophils were incubated with glycosidase for 15 min at 37°C before the addition of stimulant.

Effects of ConA on gonococcus-induced neutrophil CL. The data presented above suggested that a mannose-specific, lectin-like component was located on P.II$^+$ gonococci and that its mannose-containing receptor was located on the neutrophil. To further test this hypothesis, we preincubated neutrophils for 15 min at 37°C with 0 to 500 μg of the mannose-specific lectin ConA per ml or 0 to 100 μg of the monovalent suc-ConA per ml, allowed the lectin-induced CL to plateau, and immediately added untreated gonococci. For these experiments, data are expressed not as percentage of control, but rather as peak CL for ConA and for subsequently added gonococci, to indicate the level of CL induced by lectin alone and to best indicate the subsequent increment of CL (or lack of such) induced by gonococci (Table 3). ConA at 300 and 500 μg/ml essentially abrogated subsequent stimulation by gonococci, suggesting that all receptors were occupied by ConA. Concentrations of ConA below 10 μg/ml did not substantially inhibit subsequent gonococcus-induced CL (data not shown). Pretreatment of neutrophils with 100 μg of ConA per ml had no effect on subsequent neutrophil CL induced by 1 μg of phorbal myristate acetate (PMA); i.e., peak CL values were the same whether or not neutrophils were pretreated with ConA (control peak CL = 288,030; ConA-pretreated peak CL = 289,605; n = 2). These data suggested a mannose-specific neutrophil response that was specific for the gonococcus.

Effect of an anti-P.II monoclonal antibody on gonococcus-induced neutrophil CL. Only P.II$^+$ (not P.II$^-$) gonococci induced neutrophil CL (Fig. 1; 38; manuscript in preparation). To indicate a more direct role for P.IIs in the observed stimulation of neutrophil oxidative metabolism, P.II$^+$ variants of *N. gonorrhoeae* F62 and FA1090 were used to stimulate neutrophil CL after their incubation with a monoclonal antibody directed against P.IIb of strain FA1090. Gonococci were incubated with various concentrations of monoclonal antibody in HBSG at 37°C for 15 min before initiation of CL by their addition to prewarmed untreated neutrophils. The anti-P.IIb monoclonal antibody inhibited CL induced by FA1090 P.IIb in a dose-dependent manner, with a 1:10^3 dilution inhibiting CL by >90% (Table 4). The same monoclonal antibody, at a final dilution of 1:10^2, did not inhibit CL induced by a P.II$^+$ variant of F62, whose P.IIs are antigenically unrelated to those of FA1090 P.IIb (Table 4), indicating the specificity of the effect of the monoclonal antibody. Conversely, nonspecific ascites fluid, at final dilutions ranging from 1:10^2 to 1:10^5, did not inhibit CL induced by FA1090 P.IIb (98 ± 7% of control without ascites; n = 5), indicating that ascites fluid, per se, did not inhibit neutrophil CL.

The above results indicated that the anti-P.IIb monoclonal antibody specifically inhibited the ability of strain FA1090 P.IIb to stimulate the neutrophil oxidative burst. An alternative explanation for the decreased CL observed with monoclonal antibody-treated gonococci would be the cross-linking and subsequent clumping of gonococci by the monoclonal antibody. This could effectively decrease the number of gonococcal particles available to stimulate neutrophil CL. To see whether gonococci were being clumped by the monoclonal antibody, untreated gonococci (10^8 in 200 μl of HBSG) or gonococci treated for 15 min at 37°C with a 1:10^2 dilution of monoclonal antibody were gently spread on a glass microslide, dried, and Gram stained. Five hundred clumps each of treated and untreated gonococci were then counted by oil-immersion light microscopy. No clumping of gonococci owing to monoclonal antibody was observed (Table 5).

TABLE 3. Inhibition by ConA and suc-ConA of neutrophil CL subsequently induced by *N. gonorrhoeae*[a]

Inhibitor and amt (μg/ml)	Expt 1: peak CL induced by		Expt 2: peak CL induced by	
	Lectin	Gonococci	Lectin	Gonococci
ConA				
500	78,630	63,720	212,800	148,940
300	38,470	34,840	73,740	70,360
100	14,580	97,700	24,000	100,520
0		297.450		344,520
Suc-ConA				
100	2,500	119,000	2,200	241,480
10	1,500	287,000	1,700	200,300
0		204,000		304,040

[a] Neutrophils were incubated with ConA or suc-ConA, allowed to reach peak CL, and then immediately stimulated with untreated gonococci, and the CL was monitored over time. The results are representative of three runs done in duplicate. Experiments 1 and 2 were performed on different days with different neutrophils. The differences in CL response between the two experiments reflect normal differences between individuals.

TABLE 4. Inhibition by monoclonal antibody of neutrophil CL induced by P.II$^+$ gonococci

Strain	Monoclonal antibody titer[a]	CL inhibition	
		No. of expts	% of control[b]
FA1090	10^2	2	1.0–19.1
	10^3	4	7.5 ± 4.7
	10^4	3	13.9 ± 2.1
	10^5	2	50.1–165
F62	10^2	2	85–105

[a] Reciprical of the final monoclonal antibody dilution used in the CL reaction vial.
[b] Data are expressed as mean ± SD when $n > 2$.

DISCUSSION

The presence of carbohydrate-containing receptors on phagocytes is well known. Isolated plant lectins such as ConA and wheat germ agglutinin have been used to define roles for mannose-, *N*-acetyl-glucosamine-, and sialic acid-containing receptors in the stimulation of the oxidative burst of human phagocytes (7, 32, 34). Several gram-negative organisms associate with and are phagocytosed by eucaryotic cells by a mechanism that is mediated by mannose-

TABLE 5. Effect of monoclonal antibody on aggregation of gonococcal strain FA1090 P.IIb

Monoclonal antibody[a]	No. of aggregates containing the following no. of gonococci[b] (%):			
	1 to 4	5 to 8	9 to 12	>12
−	453 (90.6)	46 (9.2)	1 (0.2)	0 (0)
+	460 (92)	34 (6.8)	4 (0.8)	2 (0.4)

[a] Treated (+) gonococci were incubated for 15 min at 37°C with a 1:100 dilution of monoclonal antibody, spread on a microslide, dried, Gram stained, and counted, as decribed in Materials and Methods. Untreated (−) gonococci were incubated with HBSG alone and stained as above.
[b] Number of gonococci per aggregate.

specific components located on the bacterial outer surface (2, 30, 41–43). These components generally appear to be localized on pili or at least are coexpressed with pili (29, 42, 45). Gonococcal pili act as adhesins in interactions with host epithelial cells and as hemagglutinins whose activity is apparently not blocked by mannose (22, 33).

Although pili are unquestionably important in the pathogenesis of gonorrhea, such as in adherence to epithelial cells (31, 33, 46, 57) and inhibition of phagocytosis by professional phagocytes (8, 29, 36, 53, 54), other gonococcal surface components also play important roles in each of these events (21, 38, 40, 58). The genetics, structure, and function of one of these gonococcal surface components, P.II, is being intensively studied by several investigators (3–5, 24, 49). We have shown previously that nonpiliated, opaque ($P.II^+$) gonococci are phagocytically killed by human neutrophils and monocytes in the absence of serum and that they readily induce neutrophil CL (26, 38). Recent observations have indicated that only certain P.IIs, regardless of their association with opacity, confer this "pro-phagocytic" capacity upon gonococci (manuscript in preparation).

To further investigate the role of P.II in gonococcus-leukocyte interactions, we investigated the ability of mannose and other carbohydrates to inhibit the stimulation of human phagocytes by $P.II^+$ gonococci. To our surprise, several carbohydrates substantially inhibited induction of CL. For most of these carbohydrates, including glucosamine, sialic acid, and *N*-acetyl-glucosamine, we do not yet know the directionality of the lectin-like interactions; i.e., we have not discerned whether the carbohydrate-containing receptor(s) is on the leukocyte or on the gonococcus; the same obviously holds true for the lectin-like ligand(s). Kinane et al. reported inhibition of binding of gonococci to human neutrophils by galactose, *N*-acetyl-galactosamine, galactosamine, and glucosamine and showed that the lectin-like ligands for these carbohydrates were on the neutrophil, not on the gonococcus (20); they did not study the neutrophil oxidative burst. Contrary to the results of Kinane, our present study showed no inhibition of gonococcus-induced neutrophil CL by galactose or galactosamine; however, glucosamine was the most potent inhibitor that we studied. Comparison of these two groups of data suggests that adherence and stimulation of the oxidative burst by gonococci are two separable phenomena. Indeed, current investigations in our laboratory support this concept.

Because of the recent interest in mannose-dependent bacterium-host cell interactions, we decided to study the role of mannose in more detail. As opposed to most of the mannose-sensitive bacterial surface components described in the literature, the lectin-like gonococcal surface component described in this communication is not pilus associated, because nonpiliated gonococci (colony type 3) were used for the majority of the work. The component is thus more like a mannose-specific cell surface lectin isolated from *Escherichia coli* described by Eshdat et al. (10). In support of a nonpilus, lectin-like component on the gonococcal surface, Blake recently described the lectin-like activity of gonococcal P.II (3a). His investigations were stimulated by the observation that $P.II^+$ gonococci aggregate; thus, the lectin-like qualities of P.II aid in its aggregation. Mannose appears to bind to gonococci and subsequently inhibit neutrophil CL (by 73.8%), as shown in experiments in which gonococci were incubated with 100 mM mannose, washed, and used to stimulate CL. We believe, for the following reasons, that the subsequent inhibition of CL was due to mannose bound to the gonococcus and not to mannose remaining after the wash step. The volume of 100 mM mannose remaining after one wash of the gonococci was less than 5 μl. Thus, when washed gonococci were suspended in 1 ml of HBSG before use in the CL assays, residual mannose would be ≤5 mM. If one compares the 73.8% inhibition of CL obtained for mannose-treated, washed gonococci with the 47.9% inhibition seen for CL inhibited in the presence of 5 mM mannose (Table 1), it is apparent that residual soluble mannose was not responsible for the observed inhibition. This argument is supported by the fact that neutrophils incubated with 100 mM mannose and then washed responded to untreated gonococci to the same degree as neutrophils incubated in buffer (HBSG) and then washed.

Indication of the directionality of the binding comes from experiments with ConA (Table 3). Preincubation of neutrophils with ConA (≥300 μg/ml) totally inhibited the ability of subsequently added gonococci to induce further CL. To the contrary, PMA-induced CL of ConA (100 μg/ml)-treated neutrophils was as great as PMA-induced CL by untreated neutrophils. These controls confirm the observations of Cohen and colleagues indicating that ConA does not interfere with subsequent induction of the neutrophil oxidative burst by PMA (6). In further support of a mannose-containing receptor on the neutrophil, we showed that pretreatment of neutrophils with mannosidase substantially inhibited subsequent gonococcus-induced CL; this inhibition was approximately 100-fold greater than subsequent PMA-induced CL (Table 2). On the other hand, pretreatment of gonococci with mannosidase had no effect on their ability to stimulate CL. The fact that mannosidase treatment of neutrophils did indeed inhibit PMA-induced CL, however, suggests that we might be observing two phenomena in tandem—a specific effect of mannose on gonococcus-neutrophil interactions and a nonspecific (meaning undefined) effect of mannose on leukocyte metabolism. This is supported by the fact that alpha-methylmannoside was not as effective an inhibitor of CL as was mannose (Table 1). We are currently investigating this possibility.

The lack of inhibition of gonococcus-induced CL by sialidase pretreatment is contrary to the observation of Tsan and McIntyre, who showed that sialidase pretreatment inhibited subsequent superoxide production, but not hexose monophosphate shunt activity or phagocytosis, by latex-stimulated human neutrophils (56). Although we did not measure sialic acid release from sialidase-treated neutrophils, we used the highest concentration of sialidase shown to be effective by the above authors. The fact that gonococci possess a more complex array of surface components than do latex spheres can be used to explain the differences between our results and those of Tsan and McIntyre (56). Another possibility is that sialidase treatment exposes previously "buried" carbohydrates, e.g., glucosamine or mannose, that also act as receptors for the gonococcal component(s) responsible for the observed neutrophil stimulation. Much work obviously remains to be done on this question.

We are presently studying several possibilities as to the identity of a mannose-specific lectin-like stimulatory surface component on $P.II^+$ gonococci. The stimulatory component could be (i) P.II itself; (ii) a component coexpressed with P.II; (iii) a non-P.II component associated with $P.II^-$ variants whose activity is somehow expressed when P.II is inserted into the outer membrane; or (iv) a component that is constitutive on all gonococci but stimulatory only when brought into close juxtaposition to the neutrophil surface by the presence of a strongly adhesive P.II. Although the present investigation studied only nonpiliated gonococci, the

relative roles of P.IIs and pili must be studied to best understand the interaction of gonococci with leukocytes. Pili might be able to bring a stimulatory gonococcal surface component close enough to the neutrophil membrane to stimulate CL. This is inferred from our previous observations that, in the absence of serum, piliated P.II$^-$ gonococci stimulate about as much neutrophil CL as do nonpiliated P.II$^+$ gonococci (38). The observation of Koransky et al., who showed that mannose does not inhibit hemagglutination mediated by piliated gonococci, suggests that pili may not be directly involved in neutrophil stimulation through a mannose-specific receptor (22). The monoclonal antibody data presented above, in conjunction with the recent observation by Blake that purified P.IIs have lectin-like qualities (3a), make it tempting to speculate that P.II is itself the stimulatory agent; however, further experimentation must be done. Certainly gonococcal surface components other than P.II affect host-cell function and might be responsible for our observations. Recently Wiseman et al. showed that piliated gonococci inhibit anion transport by human erythrocytes (60), and Blake and Gotschlich have shown the transfer of the gonococcal porin (protein I or P.I) to erythrocyte membranes (4).

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