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Studies on Gonococcus Infection

XV. Identification of Surface Proteins of *Neisseria gonorrhoeae* Correlated with Leukocyte Association

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Neisseria gonorrhoeae which exhibit high levels of leukocyte association have a surface protein which is considerably diminished in isogenic gonococci which exhibit low levels of leukocyte association (LA). The LA protein exhibits strain variation in molecular weight and immunogenicity. Membranes derived from LA+ and LA− organisms show quantitative differences in their adsorption to leukocytes; these differences are analogous to those found for the intact organisms regarding their association with leukocytes.

The interaction between *Neisseria gonorrhoeae* and human polymorphonuclear leukocytes (PMNs) has been investigated by numerous laboratories and has been of interest because avoidance of phagocytosis is generally considered to be important as a virulence-enhancing property of pyogenic bacteria. Several groups have examined phagocytosis of gonococci (GC) in vitro and determined that the nonpilated colony types 3 and 4 of Kellogg et al. (13) are phagocytized at a higher rate than the pilated colony types 1 and 2. They have concluded that pili influence virulence by virtue of this phagocytosis-inhibiting action (2, 6, 18, 20, 29, 30, 32). An ethylenediaminetetraacetic acid (EDTA)-sensitive factor on the surface of the GC has also been shown to have an antiphagocytic role in vitro (24).

Previously, we demonstrated that nonpilated GC can exhibit either higher or lower levels of association with human PMNs than pilated GC of the same strain (27). These findings led to the hypothesis that a nonpilus factor was the primary determiner of interaction between GC and PMNs. These GC-leukocyte interactions, called leukocyte association (LA), have been assayed by light microscopy and through the use of radiolabeled bacteria; GC showing a high level of association with leukocytes in vitro are LA+, and those with a low level are LA− (24, 26-28; G. King and J. Swanson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, D28, p. 56). Both pilated (P+) and nonpilated (P−) GC can exhibit LA+ and LA− behavior. LA of LA+ GC is drastically reduced to LA− levels by incubation of the bacteria with trypsin. If the trypsin-treated LA+ GC are washed and suspended in growth medium, their LA+ behavior returns to original levels within 2 h; chloramphenicol inhibits the reappearance of LA+ behavior in trypsin-treated LA+ organisms (28).

LA+ and LA− GC appear to differ in their interactions with eucaryotic cells only with respect to peripheral blood PMNs. Attachment to other cell types (mouse peritoneal macrophages, HeLa, amnion, and buccal mucosal) is identical for LA+ and LA− GC if their state of pilation is the same (26, 27).

In the present study, we have identified differences in the cell wall surface protein patterns between LA+ and LA− GC.

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MATERIALS AND METHODS

GC. *N. gonorrhoeae* strains MS11 and F62, as well as a fresh cervical isolate (C109), were used in this study. The derivation, identification, and propagation of these organisms have been described previously in detail (9, 14). The strains were propagated as essentially pure cultures of nonpilated (P−), light, transparent, type 4 colonies by daily passage on clear agar medium containing 1% IsoVitaleX (Baltimore Biological Laboratory, Baltimore, Md.) modified to contain half the recommended amount of Trypticase peptone (9). Incubation was at 36°C in a CO_2 incubator.

In vitro assay for LA. An in vitro assay for LA has been described previously (28). PMNs were partially purified from fresh blood of laboratory personnel by sedimenting the erythrocytes from heparinized whole blood and washing the leukocytes sequentially with ammonium chloride and medium 199 (M199; Grand Island Biological Co., Grand Island, N.Y.). The

volume of washed PMN suspension was adjusted to contain 5×10^5 PMNs/0.1 ml in M199 containing 0.01% bovine serum albumin (BSA). This volume of cell suspension was pipetted onto round cover slips (diameter, 10 mm) in petri dishes (10 by 30 mm), and the leukocytes were allowed to attach at 36°C for 30 min in a 5% CO_2 atmosphere. Unattached cells were removed by washing with M199.

GC were removed from GC agar with a swab and suspended in phosphate-buffered saline (PBS; pH 7.2) at an optical opacity (Klett-Summerson Colorimeter, blue filter) of 50 Klett units (ca. 2×10^8 GC/ml). These suspensions were then diluted to 2×10^7 GC/ml in M199 containing 0.01% BSA. The PMN-laden cover slips were overlaid with 1 ml of the suspension of GC in M199 at an input multiplicity of approximately 50 GC per leukocyte. After 15 min on a rotating platform (140 rpm) at 36°C in a CO_2 incubator, the cover slips were washed with 0.5% NaCl, air dried, fixed in methanol, and stained with 3% Giemsa (Fisher Scientific Co., Pittsburgh, Pa.). The slides were examined by phase microscopy and scored by determining the percent PMNs with which GC were associated (attached or ingested). At a GC/PMN ratio of 50:1, those cultures for which the LA was >50% were considered LA+; cultures with an LA <20% were LA−. An increase in GC/PMN ratio gave a concomitant increase in LA, as described later.

The effects of nonimmune rabbit sera on attachment of GC to PMNs were determined by incubating 0.3 ml of undiluted, heat-inactivated serum with 3.0 ml of GC (opacity, 100 Klett units) for 20 min on ice. The bacteria were washed twice in PBS by centrifugation and diluted to the concentration used for the in vitro assay in M199 without BSA. The LA assay was continued as described above, with the addition of 10^{-2} M NaF to the PMN monolayer 10 min before the addition of GC to inhibit phagocytosis.

SDS-PAGE. Sodium lauryl sulfate (SDS; British Drug House catalog no. 30176, obtained through Gallard Schlessinger)-polyacrylamide gel electrophoresis (SDS-PAGE) was done by the procedure of Laemmli (15), except that no SDS was incorporated into the gel (M. Wyckoff, R. Rodbard, and A. Chrambach, Fed. Proc. **35**:1383, 1976). The proteins were dissociated by boiling for 10 min in Laemmli final sample buffer containing 2% SDS and 5% 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.). For the electrophoresis of proteins from whole bacteria, 8 ml of the suspensions of GC in PBS with an opacity of 150 Klett units (5×10^9 colony-forming units [CFU] per ml) were centrifuged, and the pellet was suspended in 150 μl of final sample buffer. Acrylamide (*N,N'*-methylenebisacrylamide; 30:0.8; Sigma) in the separating gel had a final concentration of 12.5%; the stacking gel was 5%. Electrophoresis was run at a constant current of 40 mA in a slab gel apparatus similar to that described by Reid and Bieleski (21). Gels were stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, Calif.) by the procedure of Fairbanks et al. (3). Ovalbumin, pepsin, α-chymotrypsin, and trypsin were used as molecular-weight markers. Wet gels were scanned on an Ortec 4310 densitometer and then dried on a Hoefer Scientific Instruments gel dryer. Autoradiography was done by exposing the dried gels to RP/M X-omat mammography film (Eastman Kodak Co., Rochester, N.Y.).

Preparation of envelopes. Bacteria were lysed by passage through a cooled French pressure cell (American Instrument Co., Inc., Silver Spring, Md.; 15,000 to 20,000 lb/in^2). After centrifugation to remove the remaining intact bacteria (20,000 × *g*; 5 min), the envelopes were concentrated by ultracentrifugation (260,000 × *g*; 30 minutes). Glucose 6-phosphate dehydrogenase was assayed as an indicator of cytoplasmic contamination (17). Envelopes were separated into inner and outer membranes on a 20 to 60% (wt/wt) sucrose gradient (13).

^{125}I labeling of GC. GC were removed from agar medium with swabs after 18 to 24 h of growth and suspended in PBS to yield 20 ml with an opacity of 100 Klett units (~10^9 CFU/ml). The suspension was then centrifuged, and the bacterial pellet was ^{125}I labeled by a slight modification of the method of Marchalonis et al. (16). Lactoperoxidase was added (200 μl; 0.8 mg/ml; Sigma), as well as 200 μl of [^{125}I]Na (1.0 mCi/ml) (carrier free; New England Nuclear Corp., Boston, Mass.) in 10^{-5} M KI. The mixture was incubated at ambient temperature for 10 min with additions (25 μl each) of 0.03% H_2O_2 at 0, 2.5, 5.0, and 7.5 min. The labeled suspension was then diluted to 30 ml with PBS containing 5 mM cysteine and washed twice by centrifugation to remove free ^{125}I and lactoperoxidase.

Proteins present in salt-extracted membranes of LA+ and LA− GC. Because the preparation of envelopes and the subsequent sucrose gradient yielded only small quantities of outer membrane protein, salt extraction was used to obtain surface proteins for the in vitro experiments. MS11 LA+ and LA− GC were grown on clear medium for 20 h and were labeled with ^{125}I as described above. The labeled, washed organisms (0.6 to 0.8 g, wet weight) were suspended in 0.2 M NaCl, 0.1 M tris(hydroxymethyl)aminomethane (Tris; pH 8.0) and were incubated at 45°C in a shaking water bath for 80 min in a flask containing glass beads (diameter, 0.45 to 0.5 mm). This suspension was washed into centrifuge tubes and intact organisms were pelleted by centrifugation at 13,000 × *g* for 10 min, and this supernatant was subjected to ultracentrifugation (160,000 × *g*; 90 min). The pellet was suspended in 0.5 ml of the buffered solution noted above and was applied to a Sepharose 6B column (30 by 1.5 cm). Radioactivity was found almost exclusively in the void fractions, which were combined, and these membranes were pelleted by ultracentrifugation (160,000 × *g*; 90 min). The pellet was suspended in 0.5 ml of 0.01 M Tris, 0.01 M EDTA, 0.02% sodium azide, and 2% sodium deoxycholate (DOC), incubated at 65°C for 2 h and at 36°C overnight, and blended in a Vortex mixer. This suspension was centrifuged in an Airfuge (Beckman Instruments, Inc., Fullerton, Calif.) at 30 lb/in^2 (136,000 × *g*) for 90 min. The supernatant was carefully removed and applied to a Sephadex G150 column (114 by 0.95 cm), equilibrated with 0.01 Tris, 0.01 M EDTA, and 0.2% DOC (pH 8.0), and eluted with the same buffer (6). Fractions (ca. 0.8 ml) were collected, and radioactivity was estimated in a Biogamma counter (Beckman Instruments). Proteins were precipitated by acetic acid at pH 4.5.

In vitro assay for LA activity of extracted membrane-bound proteins. Leukocytes were prepared as previously described, except that BSA was omitted from the medium and NaF (10^{-2} M) was added to inhibit phagocytosis. ^{125}I-labeled extracts concentrated by ultracentrifugation and suspended in 100 μl of PBS and equal volumes of unlabeled LA− extracts were added at various concentrations to 1 ml of M199 containing 1.5×10^8 leukocytes. The suspension was incubated on a rotator (8 rpm) at 36°C for 10 min. The leukocytes and associated proteins were removed by centrifugation (5000 × *g*; 5 min). The pellet was washed with PBS twice by centrifugation and the ^{125}I was counted in a Beckman Biogamma gamma counter equipped with a preset window for ^{125}I. For electrophoresis, the pellet was resuspended in 15 μl of Laemmli sample buffer and boiled for 10 to 15 min.

Antibody production. Whole LA+ MS11 bacteria were solubilized as described previously, and 100 μl was subjected to electrophoresis on an SDS-polyacrylamide slab gel (13 cm wide). After fixation, staining, and decolorization of the gel, that portion containing the 29,000 (29K)-dalton protein band was excised from the gel, mashed in 0.5 ml of water, eluted for 2 days at ambient temperature, and mixed with 0.5 ml of complete Freund adjuvant. This acrylamide slurry in adjuvant was injected directly into the surgically exposed inguinal lymph nodes of a 2.2-kg rabbit (7). The rabbit received an intramuscular injection of equivalent material 3 weeks later. A second intramuscular injection 5 weeks after the initial injection consisted of the same excised SDS-PAGE Coomassie brilliant blue-stained band mixed with 0.03 ml of keyhole limpet hemocyanin and glutaraldehyde at a final concentration of 0.2% to increase the antigenicity of the protein (1). The rabbit was bled thrice at weekly intervals beginning 2 weeks after the second booster. This serum was pooled and inactivated at 56°C for 30 min.

Normal rabbit serum from the same rabbit was obtained one day before the initial injection and was heat inactivated as described above.

The serum was characterized by a standard microcomplement fixation test (32). A frozen stock of whole GC, heat killed at 60°C for 30 min and with an opacity of 25 Klett units, was used as the antigen. Complement was stored at −70°C and tested for activity before each assay.

Agglutination was done in microtiter plates (Falcon Plastics, Oxnard, Calif.) with 100 μl of bacteria (50 Klett units; blue filter) in PBS with 2 mM EDTA and 100 μl of serially diluted serum per well. Bacteria in the absence of serum were examined to determine background levels of agglutination. The serum was adsorbed by incubating 300 μl of heat-inactivated serum with 25 μl of a frozen stock of LA− GC (5×10^{10} GC/ml) for 30 min at 4°C. The bacteria were removed by centrifugation, and the adsorption was repeated until there was no further change in the agglutination or complement-fixing activity.

Immunodiffusion was done in 1% agarose with 2% polyethylene glycol (molecular weight, 6K) in PBS. Salt-extracted membranes which had been dialyzed against PBS were used as the antigen. The slides were incubated for 24 h in a moist chamber, washed in saline for 48 h, and stained with 0.2% Coomassie brilliant blue in 25% isopropanol, 8% acetic acid for 2 min, and then destained in 25% isopropanol, 8% acetic acid.

Metaperiodate treatment. Bacteria (opacity, 50 Klett units) were incubated with 10 mM sodium metaperiodate (gift of J. T. Baker) on ice for 20 min before being added to the in vitro LA assay. Periodate-treated GC were not washed, since preliminary experiments indicated no difference whether or not periodate remained in the reaction mixture. This reagent cleaves the C—C bond between vicinal hydroxyl groups of sugars. The input of the LA− bacteria was increased 10× to magnify any effect of periodate to a visible level. Controls were subjected to the same incubation in the absence of periodate. The same procedure was used for the treatment of salt extracts.

RESULTS

SDS-PAGE of whole gonococcal lysates. Nonpilated, transparent colony forms of strains MS11, F62, and C109 that exhibit LA+ behavior were compared with isogenic organisms with similar colony characteristics and LA− reactivity with PMNs in vitro. In each strain, the patterns seen by Coomassie blue staining through comparative SDS-PAGE were identical for LA+ and LA− organisms, except for one band which was more intense or seen only in the LA+ preparations. In strains MS11 and F62, both of which have major outer membrane proteins (MOMPs) of 34K molecular weight, the LA+ associated band had an apparent molecular weight of 29K (Fig. 1 and 2); in strain C109, the molecular weight of the MOMP is 32K, and the LA+ associated band is 28K (Fig. 4). There does not appear to be a clear, strict relationship between the molecular weights of MOMP and the LA protein (LAP); on three separate occasions, the molecular weights of MOMPs of strains MS11 and F62 have undergone shifts (34K to 32K) during daily serial passage and periodic SDS-PAGE of these organisms. In all instances, although the molecular weights of the MOMPs changed, the apparent size of the LAP (29K) remained the same.

The differences in protein patterns of LA+ and LA− organisms were also found for strains MS11, F62, and C109 when whole GC were ^{125}I-labeled by the lactoperoxidase method, solubilized in SDS, and subjected to SDS-PAGE, and autoradiograms were developed from the resultant gels. In strain MS11 (Fig. 3) and in strain C109 (Fig. 4), the respective 29K and 28K LAP bands are heavily radiolabeled, as seen by comparing the Coomassie blue staining patterns (Fig. 3A and 4B) with those found by autoradiography (Fig. 3C and 4D).

Adsorption of salt extracts of GC to PMNs. Membranes pelleted from salt extracts

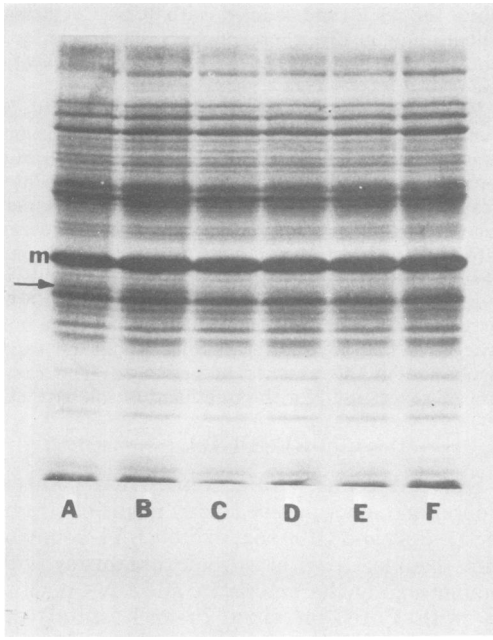

FIG. 1. *A number of cultures of LA+ and LA− GC derived from strain MS11 were obtained and serially passaged for several months. By SDS-PAGE, the LA+ organisms (lanes A and B) have a protein band (arrow) not found in GC that were LA− (lanes C-F). The major outer membrane (m) and all other visible bands are identical in all these isogenic preparations.*

of whole LA+ GC adsorbed to leukocytes more avidly than membranes from similar extracts of whole LA− organisms (Table 1). This enhanced adsorption of LA+ membranes mirrored the behavior of whole, intact LA+ and LA− GC. After incubation of these membranes from strain MS11 LA+ organisms with PMNs, three ^{125}I-labeled proteins were found associated with the leukocytes (34K, 29K, and 28K; Fig. 5B). With strain C109 LA+ as the source of the extract, two bacterial proteins were found associated with the leukocytes (32K and 28K; Fig. 5D). In PMN membrane mixtures prepared from ^{125}I-labeled LA− GC (MS11 or C109), only a faint band corresponding to the respective MOMPs was seen (not shown). Prolonged exposure of the autoradiograms showed no additional ^{125}I-labeled proteins in the leukocyte-bound material.

Separation of the LA protein from the MOMPs. The void volume of a Sepharose 6B column contained the majority of the outer membrane proteins, including the LA protein as indicated by the ^{125}I label. This complex of proteins which contained 20% of the recoverable succinic dehydrogenase activity was separated into two peaks in the presence of 0.5% DOC on a G150 column. The first peak contained primarily the MOMP; the second peak contained the LA protein as well as a 28K protein (Fig. 6). The proteins were quite insoluble after the removal of DOC and could not be tested in the in vitro leukocyte assay.

The LAP banded with MOMP and other ^{125}I-labeled proteins on an isopycnic sucrose gradient (density, 1.210 g cm^{-3}).

Assessment of antiserum to LAP. LAP antiserum raised by use of the MS11 29K LAP band excised from SDS-PAGE gels as immunogen was assessed by complement fixation by using whole bacteria as target antigen. There was a slightly higher titer with the MS11 LA+ antigen (1:32) than with the MS11 LA− antigen (1:8). All of the complement-fixing activity was removed by repeated adsorption of the serum with MS11 LA− bacteria. There was no complement-fixing activity with normal serum or when C109 was used as the target antigen with this LAP antiserum. The agglutination assay was similar in that it showed a fourfold higher titer with the MS11 LA+ organisms than with the MS11 LA− GC. By immunodiffusion, no precipitin lines were observed on reaction of this antiserum with salt extracts of either LA+ or LA− preparations of C109 GC. Extracts of MS11 LA+ and LA− which contained comparable amounts of protein showed a quantitative difference in reactivity with the MS11 LA+ antiserum. Two precipitin lines were seen which showed identity

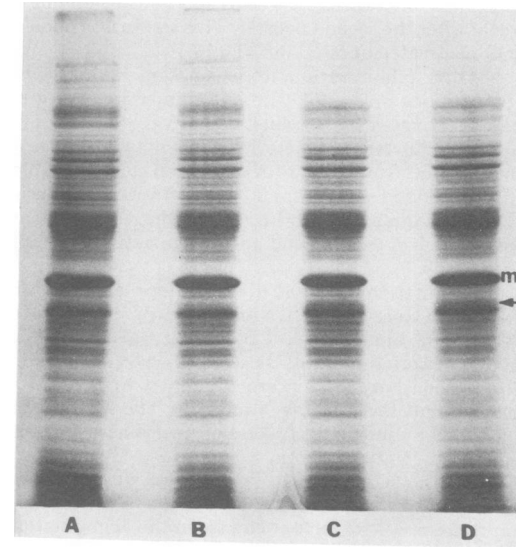

FIG. 2. *Derivatives of strain F62 were solubilized in 2% SDS with β-mercaptoethanol and subjected to SDS-PAGE (15). (A, B) LA−; (C, D) LA+. Arrow points out LA protein in lanes (C) and (D). m, Major outer membrane protein.*

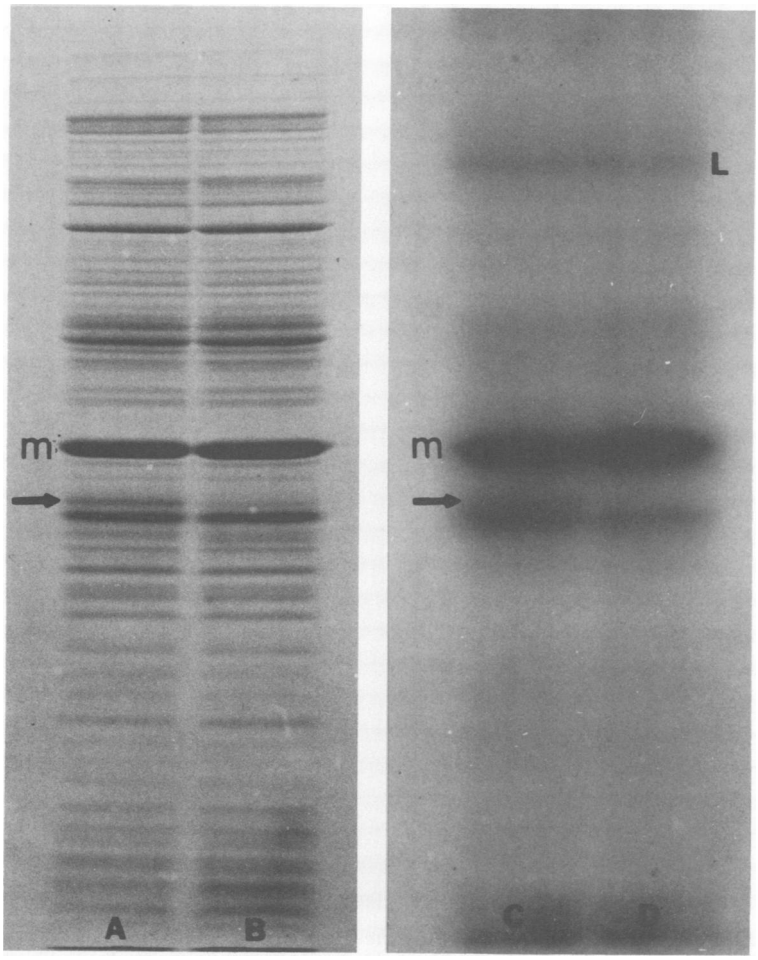

FIG. 3. *Derivatives of strain MS11 ^{125}I labeled by the lactoperoxidase-H_2O_2 catalyzed reaction (16) were subjected to SDS-PAGE and stained with Coomassie blue (left), then exposed to X-ray film (right). Lanes A and C, LA+; lanes B and D, LA−. Note that the LAP (arrow) is labeled with ^{125}I. L, Residual lactoperoxidase.*

between MS11 LA+ and LA− wells (Fig. 7). Both of these lines appear more prominent with LA+ extracts than with LA− ones, suggesting that the LA− bacteria possess smaller quantities of the LAP than do the LA+ bacteria.

Effect of LAP serum on LA. In the presence of 1×10^{-2} M NaF and the absence of serum, LA− cultures had an LA level of less than 1%. Any LA occurring under these conditions was assumed to result from attachment of GC to PMN surfaces, since phagocytosis had been inhibited. Under these conditions, the LAP serum prepared against the MS11 29K LA+ SDS-PAGE band significantly reduced the level of association of MS11 and F62 LA+ bacteria with PMNs (Table 2). Normal rabbit serum only slightly reduced GC-PMN association. The MS11 LAP serum had no effect on LA of strain C109.

Effect of periodate on LA. When the bacteria were incubated with sodium metaperiodate before the in vitro assay, LA of LA+ bacteria was markedly reduced (Table 3). The LA− bacteria showed no discernable change in LA. Similar results were observed when salt extracts were treated with periodate (Table 4). Pretreatment of the leukocytes with periodate had no effect (data not shown). These results suggested that a carbohydrate moiety may be important in the GC-PMN interaction. Accordingly, blocking the GC-PMN association by simple sugars (mannose, galactose, fucose, and xylose; 23 mg/ml) was attempted. Neither these sugar additions nor pretreatment of LA+ GC with glycos-

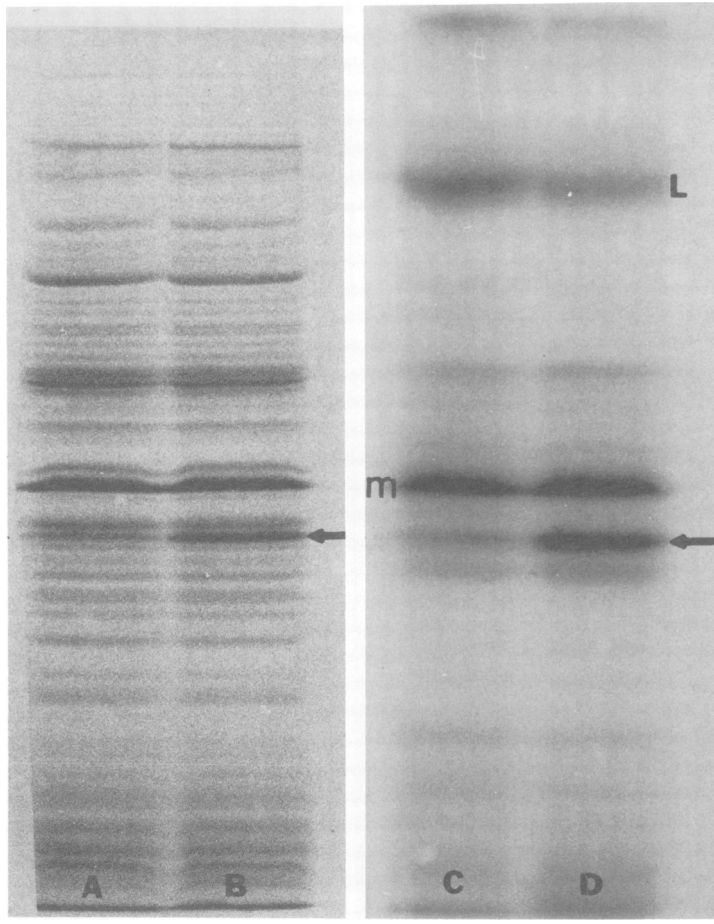

FIG. 4. *Derivatives of strain C109 ^{125}I labeled by the lactoperoxidase-H_2O_2 catalyzed reaction (16) were subjected to SDS-PAGE and stained with Coomassie blue (left), then exposed to X-ray film (right). Lanes A and C, LA−; B and D, LA+. Note that the LAP (arrow) is ^{125}I labeled. L, Residual lactoperoxidase.*

idases (xylosidase, β-glucosidase, glucuronidase, β-galactosidase, fucosidase, and mannosidase; 0.4 to 5 mg/ml) resulted in changes in GC-PMN association (data not shown).

Stability of LA characteristics. In the absence of a selective medium, it is difficult to accurately determine the rate of change from LA+ to LA−. Crude approximation of such a rate was made by subculturing single colonies and testing the subsequent cultures for LA. On subculturing 150 colonies from LA+ MS11 culture, 12 of the resultant cultures were LA−. A similar percentage (8%) of cultures were LA− on subculturing 60 single colonies from an LA+ preparation of strain C109. In all cases, the change in in vitro behavior was accompanied by the loss of the LAP band in the SDS gels.

The change from LA− to LA+ appears to have occurred at a much lower frequency. We have isolated only two LA+ cultures from MS11 LA− GC during the course of this study. Both of these isolates contained the LAP (molecular weight, 29K).

TABLE 1. *Adsorption of ^{125}I-labeled gonococcal extracts to leukocytes*

Strain	Phenotype(s)	% Total ^{125}I absorbed to leukocytes[a]
MS11	LA+	3.0 ± 0.15
MS11	LA−	0.7 ± 0.2
MS11	LA+	3.2 ± 0.4
MS11	LA− (unlabeled)[b]	
C109	LA+	1.7 ± 0.2
C109	LA−	0.6 ± 0.2

[a] Values represent mean ± standard error from three separate experiments.

[b] Unlabeled LA− extracts were mixed with labeled LA+ extracts before addition to leukocytes.

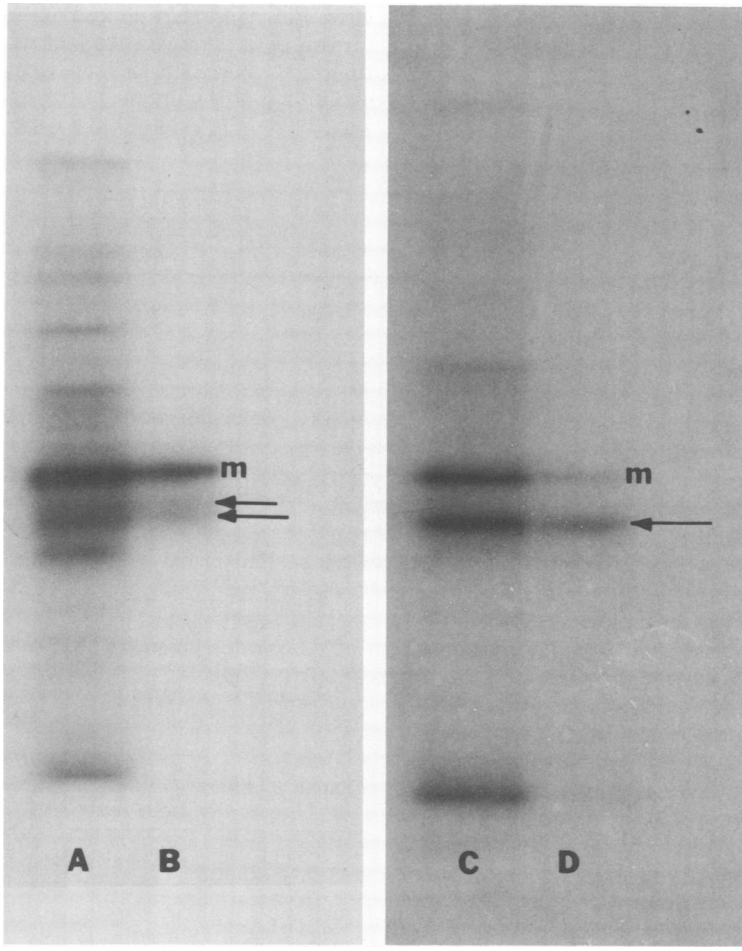

FIG. 5. *^{125}I-labeled membrane extracts were mixed with unlabeled leukocytes on a rotator for 10 min, and then the leukocytes and associated gonococcal proteins were removed by centrifugation. Whole extracts and the leukocyte-containing pellet were subjected to SDS-PAGE, and the gel was then exposed to X-ray film. Autoradiograms: A, strain MS11 extract; B, proteins from MS11 extract which associated with the leukocytes; C, strain C109 extract; D, proteins from C109 extract which associated with leukocytes. m, MOMP. Note that with strain MS11 primarily two proteins (arrows), the LAP and a slightly smaller protein, in addition to the MOMP were found associated with the leukocytes. With strain C109, there was primarily only one protein, the C109 LAP (arrow), which, with the MOMP, was found associated with the leukocytes.*

DISCUSSION

Whether a bacterium will show attachment to and ingestion by phagocytic leukocytes depends, in part, on the characteristics of the surface of the microorganism. This has been demonstrated by several methods, including the studies of Van Oss and Gillman (31), which correlated the degree of bacterial surface hydrophobicity with the degree to which the organisms underwent phagocytosis in vitro. Of the several bacterial surface components that have been studied, emphasis has been placed on those which diminish or restrict uptake of bacteria by PMNs or macrophages. This stems from the fact that effecting a diminution in phagocytosis is generally considered to abet survival of the bacteria in vivo and is therefore viewed as a virulence factor of these organisms. Well-known examples of such surface virulence factors that negatively influence phagocytosis of bacteria include streptococcal M-protein and pneumococcal or anthrax bacillar capsules (4, 19).

Several surface components of *N. gonorrhoeae* have been reported to exert negative influences on phagocytosis of GC by PMNs in

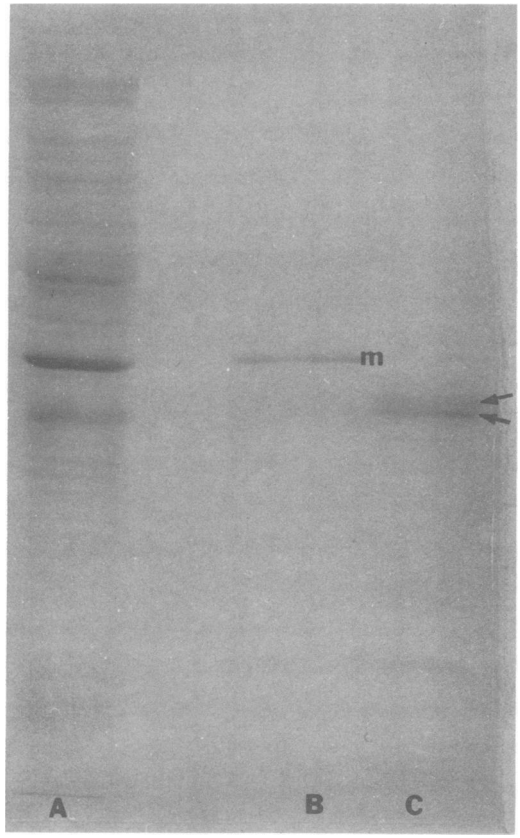

FIG. 6. *NaCl extract of LA+ derivative of strain MS11 was fractionated on a Sepharose 6B column, and the material in the void volume was concentrated and passed over a Sephadex G-150 column with 0.2% DOC. The extract (A), peak 1 (B), and peak 2 (C) from the G-150 column were subjected to SDS-PAGE. Note the MOMP (m) is in peak 1, whereas the LAP and a slightly smaller protein (arrows) are the principal components in peak 2.*

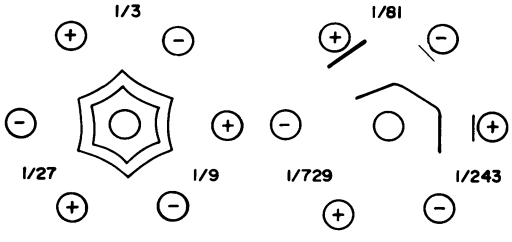

FIG. 7. *NaCl extract of LA+ (⊕) and LA− (⊖) derivitives of strain MS11 at dilutions from 1:3 to 1:729 were subjected to immunodiffusion with MS11 LAP serum (center wells). There were two distinct precipitin bands which showed identity between the LA+ and LA− wells, but both bands were stronger with the LA+ antigen than with the LA− antigen.*

vitro. Pili, capsules, and an EDTA-sensitive factor are putative virulence factors for GC, since they are envisioned as causing reduced uptake and killing of the organisms by leukocytes (2, 8, 18, 22, 23, 30). Others have suggested that, al-

TABLE 2. *LA of LA+ GC in the presence of LA+ MS11 serum under conditions inhibiting phagocytosis*

Strain	Serum	% Leukocytes with associated GC[a]
MS11[b]	None	35 ± 0.3
MS11	Normal	21 ± 0.1
MS11	Immune (LA specific)[e]	9 ± 0.3
F62[c]	None	30 ± 0.4
F62	Normal	25 ± 0.2
F62	Immune	9 ± 0.2
C109[d]	None	22 ± 0.4
C109	Normal	15 ± 0.3
C109	Immune	13 ± 0.2

[a] Values represent mean ± standard error of four separate experiments.

[b,c,d] LA in the absence of NaF was 94%, 90%, and 68%, respectively.

[e] Serum was prepared by using the 29K protein of MS11 from an SDS-polyacrylamide gel as immunogen.

TABLE 3. *Effect of metaperiodate on LA of strains MS11 and Cl109*

Strain	Periodate[a]	% Leukocytes with associated GC[b]
MS11-LA+	−	92
MS11-LA+	+	41
MS11-LA−[c]	−	48
MS11-LA−[c]	+	51
C109-LA+	−	60
C109-LA+	+	25
C109-LA−[c]	−	30
C109-LA−[c]	+	32

[a] +, Periodate added; −, no periodate added.

[b] Data from one of four separate experiments are presented.

[c] The input multiplicity of the LA− cultures was increased 10× to magnify any effect of the metaperiodate.

TABLE 4. *Effect of sodium metaperiodate on adsorption of ^{125}I-labeled bacterial extracts of MS11 to leukocytes*

Phenotype	Periodate[a]	% Total ^{125}I adsorbed to leukocytes[b]
LA+	−	3.8
LA+	+	0.7
LA−	−	0.8
LA−	+	0.8

[a] +, Periodate added; −, no periodate added.

[b] Data from one of three experiments are presented.

though they are ingested, pilated GC resist intraphagocytic killing by unknown mechanisms (6, 29, 33).

Although we were also able to demonstrate differences in the LA behavior (attachment plus phagocytosis) of pilated versus nonpilated GC and of EDTA-treated versus untreated organisms, our studies have focused on the influence of another gonococcal surface component which we have previously called "leukocyte association factor." Earlier studies suggested that LA can be present or absent from both pilated (P+) and nonpilated (P−) organisms. Some GC (P+ or P−) appear to possess LA and exhibit high levels of association with PMNs in vitro; other organisms (P+ or P−) from the same strains show low or negligible association with PMNs under the same assay conditions, are thought to either lack or have small amounts of LA, and have been designated LA−. Because of its apparent susceptibility to trypsin under conditions maintaining the viability of the bacteria, gonococcal LA has been suggested to be a surface component.

Our current study extends those previous findings and suggests that LA+ and LA− GC differ with regard to the relative amount of particular proteins—LAP—on their surfaces. GC which have abundant LAP on their surface display LA+ behavior, whereas LA− organisms appear to have relatively less LAP on their surface.

The 29K (strain MS11 or F62) and 28K (strain C109) LAPs that we have observed by SDS-PAGE were clearly located on the gonococcal surface. Susceptibility to trypsin, radioiodination by lactoperoxidase labeling of whole cells, presence in membranes isolated by pressure disintegration and sucrose density gradient centrifugation, and presence in void eluate from Sepharose 6B chromatography after salt extraction of whole GC indicated a superficial location for LAP on the GC cell wall.

Direct demonstration that LAP mediates LA is not possible at present. We have been unable to maintain DOC-extracted LAP in solution in the absence of detergents, so interactions of this protein with PMNs have not been studied. DOC treatment of GC membranes solubilized LAP from the membranes while leaving MOMP in sedimentable, insoluble form. Removal of DOC from this solution rendered LAP insoluble in media we have utilized for the study of GC-PMN interactions.

The use of 29K LAP (strain MS11) as an immunogen and studies with the LAP antiserum support the idea that LA is mediated by LAP, since the anti-LAP serum significantly reduces the association between MS11 and F62 LA+ GC and NaF-treated PMNs. No effect of anti-LAP serum (against strain MS11) was seen on incubation of heterologous (strain C109) LA+ GC with the serum before their reaction with PMNs. Whether the antiserum influenced LA by coating LA+ organisms and inhibiting their attachment to PMNs or by aggregating these LA+ GC to reduce the chance of collision with PMNs is not clear.

LAP exhibited variable molecular weight depending on the strain of GC studied (29K for MS11 and F62, 28K for C109). Spontaneous, stable change in the molecular weight of MOMP has been observed in three different instances and, in each, no attendant change in the molecular weight of LAP was observed, suggesting that phenotypic expressions of these different protein moieties (MOMP and LAP) are independent phenomena. These data, along with the antigenic differences of LAP demonstrated through use of anti-LAP serum, suggest that any of several different GC surface proteins may enhance interactions of GC with PMNs and that more than one LAP species might be present on the surface of a given gonococcus. This latter suggestion is supported by preliminary studies which show comigration of 28K and 29K proteins (Fig. 6B), which are present in roughly equivalent quantities, on a G150 column (in the presence of DOC) of material obtained by salt extraction of LA+ GC (strain MS11). Whether the overall LA characteristics of the GC are influenced by the presence of both these constituents, analogous to the intergonococcal aggregating activity of several other cell surface proteins of GC, remain to be clarified.

Pretreatment of LA+ GC with periodate effected a marked reduction in LA of these GC. This was accompanied by changes in molecular weight measured by SDS-PAGE of several GC proteins, including LAP. These findings suggest that a sugar moiety is important in mediating LA of GC. We have been unable to block GC-PMN interactions with simple sugars, to reproducibly abolish LA+ behavior by assorted glycosidase treatment of intact organisms, or to demonstrate Alcian blue staining of LAP bands; so the contribution of GC cell wall or LAP saccharides to GC-PMN interactions is unclear.

Although several other GC surface components (pili, EDTA-sensitive factor, and capsules) are reported to exert a negative influence on GC-PMN interactions, LAP appears to enhance or positively affect these bacterium-host cell relationships. In our studies, the amount of LAP found on GC appears to be roughly correlated with the LA behavior of GC in the in vitro assay utilized. This in vitro assessment of GC-PMN interactions shows high correlation with the apparent in vivo association of GC with PMNs as

deduced by study of stained smears of urethral exudates from males with acute gonorrhea (14). It follows that the in vivo GC-PMN interaction should reflect the relative abundance of LAP on gonococcal surfaces. How this LAP-mediated behavior is influenced by the various phagocytosis-reducing components of the GC surface remains to be elucidated. More important for the understanding of GC pathobiology is the question of the relevance of such interactions to the virulence of GC or the pathogenesis of acute gonorrheal infections.

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