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Research Article

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Interaction of *Neisseria gonorrhoeae* with Classical Complement Components, C1-Inhibitor, and a Monoclonal Antibody Directed against the Neisserial H.8 Antigen

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Abstract

Strains of *Neisseria gonorrhoeae* were used to evaluate bactericidal and opsonic properties of McAb 10 directed against the Neisserial outer membrane antigen, H.8. Gonococci were either serum resistant in the absence but serum sensitive in the presence, of McAb 10, or serum sensitive or serum resistant regardless of the presence of McAb 10. Strain JS3, which fell in the former category, was used in subsequent studies.

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When purified classical pathway components were used to deposit C3 on JS3 in the absence of serum regulatory proteins or antibodies, added C1-inhibitor reduced C3 binding to background levels. When McAb 10 was present, C3 binding was unaffected by C1-inhibitor.

Covalently bound, large molecular weight C3α-chain-gonococcal complexes were disbanded by methylamine release of ester linkages. Released ^{125}I-C3 migrated as C3b without degradation by gonococcal proteases.

Purified classical components alone or McAb 10 alone facilitated JS3 killing by neutrophils; when combined, the two provided maximal killing. Levels of McAb 10 that only slightly increase C3 deposition on JS3 are bactericidal in serum and maximally opsonic in combination with purified classical pathway components.

Introduction

Serum bactericidal activity against *Neisseria gonorrhoeae* is primarily associated with classical complement pathway activation (1–5). Although an antibody requirement for lytic activity of serum against clinical isolates of *N. gonorrhoeae* has been suggested, this presumption has not been rigorously tested.

Selected mutants of gram-negative enteric organisms directly activate the classical complement pathway in vitro in the absence of antibody (6–9). The initiating event is conversion of zymogen C1 to its active serine esterase form (reviewed in references 10 and 11) upon interaction of C1 with constituents of the bacterial outer membrane. This activation is not abrogated by the serum glycoprotein, C1-inhibitor, if C1 interacts with rough bacteria containing (truncated) LPS, but the reaction is totally inhibited on the surface of organisms bearing smooth (complete) LPS (6, 9). *N. gonorrhoeae* contains LPS bearing only a limited number of core sugar residues (12, 13), suggesting that this organism may directly activate C1 even in the presence of C1-inhibitor.

This issue has been examined to a limited extent with gonococci during incubation in serum. Harriman and colleagues (14) showed activation of purified, radiolabeled C1s in serum during incubation with various serum-sensitive and -resistant gonococci. Shafer and co-workers (4) showed C1 consumption from agammaglobulinemic serum by a serum-sensitive mutant of *N. gonorrhoeae* with "deep rough" LPS. Nonetheless, no studies have been done with *N. gonorrhoeae* using purified complement components to exclude definitively the contribution of antibody to the observed results. Two issues thus remain unsettled: whether complement is activated in the absence of antibody and, if so, whether activation takes place in the presence of C1-inhibitor.

Recently, H.8 antigen, common to *N. gonorrhoeae* and *Neisseria meningitidis*, has been defined based on its reactivity with several independently derived MAb (15–18). H.8 is interesting for several reasons: (*a*) it has a common, antigenically invariant epitope that is immunoaccessible on viable bacteria in vitro and in vivo (16, 19–21); (*b*) the antigen (15, 16) and the gene (22) appear to be restricted to the pathogenic (and opportunistic) members of the genus; (*c*) the H.8 antigen gene contains a repetitive 15-bp sequence constituting > 90% of the structural gene (23), six copies of which are found in another virulence-associated gene, the azurin gene (24, 25); and (*d*) H.8 appears to be a lipoprotein, the first described in *Neisseria* species, and may be important in membrane architecture and integrity. Finally, the H.8 antigen is immunogenic in humans: H.8-specific antibody responses have been detected in patients with local and disseminated gonococcal and meningococcal disease (26, 27). The contribution of H.8-specific antibody to serum opsonic and bactericidal activity against *N. gonorrhoeae* is, therefore, of interest.

In this report, we studied bactericidal and opsonic activities of McAb 10, directed against the H.8 antigen. Our results indicate that McAb 10 is bactericidal for some but not all strains of gonococci during incubation in adsorbed normal human serum. We also show that the serum-resistant clinical isolate JS3 does not directly activate C1, C4, C2, and C3 independently of antibody, but rather accepts C3b deposition resulting from spontaneous, autocatalytic activation of C1. We

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demonstrate that C1-inhibitor blocks autoactivation, and that McAb 10 induces activation of C1 which is not abrogated by C1-inhibitor, thereby enhancing phagocytosis and killing of gonococci by PMN. These experiments are the first to examine rigorously the contribution of antibody to complement activation by *N. gonorrhoeae* and to examine the opsonic and bactericidal function of an H.8-reactive MAb.

Methods

Buffers and reagents. HBSS containing 1 mM $MgCl_2$ and 0.15 mM $CaCl_2$ (HBSS++)[1] was the standard buffer in these experiments. In some cases, 0.1% gelatin (Sigma Chemical Co., St. Louis, MO) or 1% BSA (immunoglobulin- and fatty acid-free, Boehringer Mannheim Biochemicals, Indianapolis, IN) was added to HBSS++ (HBSS++GEL; HBSS++ALB). All reagents were brought to room temperature before use.

Serum. Pooled normal human serum (PNHS) was obtained from five normal volunteers with no history of previous gonococcal infections. Serum was adsorbed twice against homologous glutaraldehyde-fixed *N. gonorrhoeae* to remove natural antibody, as previously described (28). Serum adsorbed by this procedure lost < 25% of hemolytic C3 activity measured as described (29). Aliquots were stored at −70°C.

Bacteria. Gonococcal strains used in these experiments are listed in Table I. All strains were tested in the serum bactericidal assays; JS3 was studied in all experiments.

Bacteria were grown on gonococcal agar base (National Institutes of Health Media Room) with 1% Isoyitalex (Becton Dickinson & Co., Inc., Cockeysville, MD) in candle extinction jars at 37°C. Transparent, nonpiliated colonies were passed daily. Colony phenotype was determined using the criteria of Swanson (30). For experiments, the bacteria were grown 18–20 h, then suspended in HBSS++GEL or HBSS++ALB at room temperature to a concentration of 1×10^8 bacteria/ml (OD_{600} = 0.100 nm).

MAb. MAb 10 directed against Neisserial surface antigen H.8 was prepared as described by Hitchcock et al. (16). Tissue culture supernatant was concentrated 20-fold using a 100-ml cell and PM100 membrane (Amicon Corp., Danvers, MA). A second sample was prepared by growing the monoclone in defined medium containing [^{35}S]-methionine from New England Nuclear, Boston, MA. The culture supernatant fluid was replaced with fresh medium to which 300 μCi of [^{35}S]L-methionine was added. The culture was incubated at 37°C in an atmosphere of 10% CO_2 for 48 h. The cell-free supernatant fluid containing the intrinsically labeled McAb 10 was harvested and used undiluted in experiments in which antibody binding was measured.

Complement components. Guinea pig C1 (GPC1; 10,000 U/ml) was purchased from Cordis Laboratories, Miami, FL. Human C1 subunits C1q, C1r, and C1s were purified using the methods of Tenner et al. (31), Ziccardi and Cooper (32), and Valet and Cooper (33), respectively. 5 μl C1q (2.7 mg/ml), 34 μl C1r (187.5 μg/ml), and 8 μl C1s (806 μg/ml) were mixed on ice in 200 μl of 1% ultrapure BSA in distilled water, 100 μl of 0.05 M $CaCl_2$, and 815 μl HBSS++GEL. The mixture was incubated at 30°C for 15 min with shaking to allow association of C1 subunits. This mixture (150 μl/tube) was used in the assays to achieve a final concentration of complement components equivalent to 5% normal serum concentration. Functionally active human C3 and C4 were purified using the procedure of Hammer et al. (34), with minor modifications. C3 was radiolabeled with ^{125}I (^{125}IC3) using Iodobeads from Pierce Chemical Co., Rockford, IL. C1-inhibitor was isolated according to previously published methods (35). Human C2 was prepared by a new procedure using polyethylene glycol precipitation, C4b/iC4b Sepharose, and DEAE Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) (36). All proteins were biochemically pure as assessed by SDS-PAGE and immunoelectrophoresis except C4, which contained small amounts of minor contaminants, but was functionally pure for C4 hemolytic activity.

Neutrophils. Neutrophils were obtained from normal volunteers with no history of gonococcal infection and who were not taking antibiotics. Lymphocyte Separation Media from Litton Bionetics, Inc. (Charleston, SC) was layered under heparinized blood diluted vol/vol with PBS, then centrifuged for 20 min at 1,200 rpm at 20°C. Plasma, platelet, and mononuclear cell supernatant was discarded. Neutrophils were sedimented with 3% dextran 500 from Pharmacia Fine Chemicals in PBS, pH 7.4, for 30 min at 22°C. Leukocyte-rich supernatant fluid was centrifuged at 1,500 rpm at 4°C. Contaminating erythrocytes were lysed with cold 0.2% NaCl and physiological ionic strength restored by addition of an equal volume of 1.6% NaCl. After the final centrifugation, the neutrophils were resuspended in HBSS++ALB at a concentration of 5×10^6/ml.

Serum bactericidal assay. Bacteria were suspended in HBSS++ALB to OD_{600} = 0.100 as described above. For presensitization, 100 μl of this suspension was mixed with 50 μl of dilutions of McAb 10 in buffer, 50 μl of an irrelevant MAb or 50 μl of buffer alone, and the suspension was incubated for 20 min at 22°C with intermittent agitation. Then 100 μl of this mixture containing presensitized organisms was added to 100 μl of 20% adsorbed PNHS in HBSS++ALB, and the suspension was incubated at 37°C for 60 min. Viable bacteria were enumerated as CFU by making serial dilutions in buffer and plating aliquots on chocolate agar plates (National Institutes of Health Media Room) for growth in candle jars at 37°C for 36–48 h. Control samples were handled identically except that presensitized organisms were mixed with 20% adsorbed PNHS previously heated at 56°C for 30 min to block complement activation. Log_{10} kill was calculated for organisms presensitized with McAb 10 or with buffer as previously described (5).

Binding of ^{35}S-labeled McAb 10 to JS3. Binding of McAb 10 to strain JS3 was examined in a quantitative binding assay. Tissue culture supernatant fluids, unlabeled and ^{35}S-intrinsically labeled, were mixed in HBSS++GEL. The mixture contained 200 μl ^{35}S McAb 10 and increasing amounts of unlabeled McAb 10 ranging from 0 to 320 μl. Gonococci, strain JS3 (1×10^6 in 100 μl HBSS++GEL), were added, and the mixture incubated at 37°C, tumbling for 1 h. Bacteria were then washed twice with HBSS++GEL. After the final centrifugation, the bacterial pellet was solubilized in 500 μl of 1% SDS by heating at 100°C for 10 min. Samples were then added to scintillation fluid from Hydrofluor; National Diagnostics, Somerville, NJ, and bacteria-associated radioactivity was counted in a liquid scintillation analyzer (2000CA Tri-Carb; Packard Instrument Co., United Technologies, Downers Grove, IL). Results are expressed as counts per minute bound with each concentration of unlabeled McAb 10.

Activation of C1 by JS3. We next examined whether JS3 directly activated C1 in the absence of antibody. Deposition of radiolabeled C1s within C1 was measured on JS3 in the presence or absence of C1-inhibitor. C1q (8 μg), C1r (4.5 μg), and C1s (4.03 μg unlabeled and 0.47 μg ^{125}I labeled) were mixed at 0°C with 15 μl veronal buffer, 0.05 M CaCl2 (final concentration 2.5 mM Ca^{2+}), and 48 μl H_2O containing 1 mg/ml ovalbumin from Sigma Chemical Co. C1-inhibitor (13.5 μg) was added to the above mixture before aliquots were mixed with JS3 (1×10^7, 5×10^7, and 1×10^8 organisms). Positive control tubes contained instead of JS3, heat-aggregated human IgG (63°C, 15 min, 1.16 mg/ml) or *E. coli* J5 (5×10^7 and 1×10^8 organisms), both of which directly activate C1 in the presence or absence of C1-inhibitor (6, 7, 37–39). Negative control tubes contained C1 activator components only in the absence of C1-inhibitor. These mixtures were incubated at 30°C for 10 min, and the reaction stopped by addition of SDS-urea-DTT (40). Samples were heated to 37°C for 45 min and SDS-PAGE (8%) and autoradiography were performed.

Classical complement pathway activation by JS3. Deposition of C3 on strain JS3 was measured during incubation with purified classical pathway components. The final concentration of each component was

1. *Abbreviations used in this paper:* GPC1, guinea pig C1; HBSS++, HBSS containing 1 mM $MgCl_2$ and 0.15 mM $CaCl_2$.

adjusted to 5% of the antigenic level (for biochemically pure proteins) or 5% of the hemolytic level (for functionally pure components) of the component in serum. Gonococcal strain JS3 in HBSS++GEL (400 μl, 1×10^8 organisms/ml) was mixed with 20 μl HBSS++GEL or 20 μl McAb 10. The mixture was tumbled end over end at 12 rpm (Rototorque, model 7637; Cole-Parmer Instrument Co., Chicago, IL) at 37°C for 10 min. GPC1 (15,000 U), human C1 (6.8 μg in 150 μl of HBSS++GEL, 0.005 M $CaCl_2$, and 1% BSA) or C1 mixed with C1-inhibitor (12.5 μg) were added to JS3 and the mixture incubated at 30°C for 15 min with shaking. The mixtures were centrifuged at 12,500 *g* (Eppendorf centrifuge) for 5 min and the supernatant fluid was discarded. Then classical complement components were added as unlabeled C3 (11.96 μg), ^{125}I-C3 (7.82 μg), C4 (6,000 U), and C2 (0.8 μg), the final volume was brought to 1 ml with HBSS++GEL, and the mixture tumbled for 15 min at 37°C. Triplicate 200-μl aliquots were transferred to microcentrifuge tubes containing 1 ml cold HBSS++GEL and centrifuged at 12,500 *g* for 10 min at 4°C. After discarding the supernatant, the sides of the tube were washed with 500 μl HBSS++GEL, and the pellet was centrifuged again, the supernatant discarded, and the pellet was counted in a gamma scintillation counter from Packard Multi-Prias, Packard Instrument Co. The bacterial pellet was solubilized in SDS sample buffer (41) containing 2% mercaptoethanol for 10 min at 100°C, then frozen at −70°C for subsequent examination by SDS-PAGE.

Form of C3 bound to JS3. The molecular form of ^{125}I-C3 deposited on the gonococcal surface with purified classical pathway components was examined. Solubilized samples from the C3 binding experiment were divided in two equal aliquots. To cleave covalent ester linkages between C3 and acceptor molecules, one aliquot was mixed with 4 μl 200 mM methylamine in carbonate buffer at pH 11 and incubated at 37°C for 1 h. Then 1.4 μl of 0.5 N HCl was added to achieve a final pH 7.8. Aliquots of methylamine- and nonmethylamine-treated samples were applied to a 7.5% SDS polyacrylamide gel and electrophoresis was performed (41). Autoradiography was done on dried gels.

Killing of JS3 by PMN. Strain JS3 was presensitized with McAb 10 and/or classical complement pathway components exactly as described above in C3 binding assays. Gonococci (1×10^7 organisms in 100 μl HBSS++ALB) bearing complement components, McAb 10, or both, were mixed with 2.5×10^6 PMN in 500 μl HBSS++ALB or HBSS++GEL (JS3/PMN = 4:1) in microcentrifuge tubes. The volume was brought to 1 ml with HBSS++ALB, and the mixture was tumbled at 12 rpm at 37°C. Samples were removed at 0, 15, 30, and 60 min, diluted serially in HBSS++GEL or HBSS++ALB, and duplicate 50-μl aliquots of each dilution were plated on chocolate agar. CFU were counted on each plate after incubation for 16–20 h in candle jars at 37°C. Results at each time period are expressed as percent of the time zero inoculum.

Results

Bactericidal activity of McAb 10. All the gonococcal strains characterized in Table I were tested for susceptibility to the bactericidal activity of adsorbed PNHS, alone or with added McAb 10 (Table II). The strains fell into three categories: (*a*) strains FA 635 and 7189 were sensitive to killing in adsorbed serum, and killing increased slightly with McAb 10 presensitization; (*b*) strains R11, FA 638, WG, 7220, 7221, and 6305 were resistant to killing in the presence or absence of McAb 10; and (*c*) strains JS3 and 7122 were serum resistant in the absence of McAb 10, but were serum sensitive when presensitized with McAb 10. In subsequent studies we used strain JS3 because of its differential susceptibility to serum killing in the presence and absence of McAb 10 and because JS3 has been well characterized with respect to outer membrane constituents (42–44).

Table I. Source and Protein I Serogroup of Gonococcal Strains

Strain	Source	Serogroup*
R11	Milton Tam	IA
FA635	Fred Sparling/Janne Cannon	IA
FA638	Fred Sparling/Janne Cannon	IA
JS3	John Swanson	IA
WG	Peter Rice	IB
6305	Leonard Mayer	IA
7189	Leonard Mayer	IB
7220	Leonard Mayer	‡
7221	Leonard Mayer	‡

* As determined by Dr. J. S. Knapp, University of Washington, Seattle, WA.
‡ Transformants made from strains 6305 and 7189, not serotyped.

Specific binding of McAb 10 to JS3. Experiments were designed to estimate the amount of McAb 10 binding to JS3. Increasing amounts of unlabeled McAb 10 were mixed with a constant amount of ^{35}S-labeled McAb 10 in buffer before gonococci were added. After 60 min at 37°C, the organisms were washed and bacteria-associated radioactivity was counted. Results were expressed (Fig. 1) as counts per minute bound at each concentration of added unlabeled antibody. Characteristic of specific binding, as increasing amounts of unlabeled McAb 10 were added, less radioactivity became associated with JS3, reflecting competitive inhibition of [^{35}S]-McAb 10 by unlabeled McAb 10. Furthermore, these results indicate that in the above experiment and in all experiments reported below using 20–50-μl volumes of McAb 10 to sensitize JS3 at 1×10^8/ml, the amounts of antibody bound are below saturating levels.

Activation of C1 by JS3. C1 zymogen was prepared by mixing C1q, C1r, and radiolabeled C1s at 0°C, with or without C1-inhibitor, before adding to C1 activators or JS3 for incubation at 30°C. No concentration of JS3 tested in the presence or absence of C1-inhibitor activated C1 as determined by absence

Table II. Bactericidal Activity of McAb 10 for 10 Strains of N. gonorrhoeae

	Log_{10} kill in 10% PNHS	
Strain	−McAb10	+McAb10
FA635 (n = 3)	2.31±0.66*	3.19±1.09
7189 (n = 3)	2.43±0.15	3.01±0.21
R11 (n = 3)	0.31±0.31	0.22±0.31
FA638 (n = 2)	0.03±0.21	−0.09±0.10
WG (n = 3)	0.01±0.03	−0.02±0.04
7220 (n = 2)	−0.01±0.06	0.13±0.06
7221 (n = 2)	0.10±0.12	0.09±0.05
6305 (n = 4)	0.06±0.09	0.07±0.08
JS3 (n = 3)‡	0.47±0.22	2.54±0.40
7122 (n = 3)‡	0.08±0.04	2.96±1.22

* Mean $\log_{10}$ kill±SD for n experiments.
‡ Strains for which $\log_{10}$ kill is significantly greater than without McAb 10.

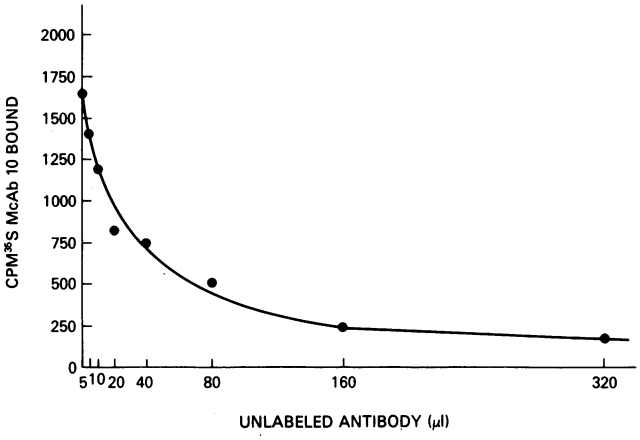

Figure 1. Inhibition of ^{35}S McAb 10 binding to JS3 by unlabeled McAb 10. Increasing volumes of unlabeled McAb 10 were mixed with ^{35}S McAb 10 and added to 1×10^6 JS3. Mixtures were rotated end over end at 37°C for 1 h, washed, and the radioactivity associated with JS3 was then measured. Unlabeled McAb 10 inhibited binding of ^{35}S McAb 10 to JS3 in a dose-dependent fashion.

of the 57-kD fragment of activated C1s on autoradiograms (data not shown). *E. coli* J5, at both concentrations tested, and aggregated human IgG produced activated C1s in the presence and absence of C1-inhibitor. These results indicate that gonococcal strain JS3 does not initiate activation of the classical complement pathway by directly activating C1 in the absence of other serum factors.

Classical complement pathway activation by JS3. We then tested whether JS3 would activate purified components of the classical pathway (C1, C4, C2, and C3) in the presence of McAb 10. Strain JS3, with or without McAb 10 presensitization, was mixed with GPC1 or reassociated human C1 subunits, followed by 5% serum levels of C4, C2, and C3 plus ^{125}I-C3 or, in control experiments, 5% serum levels of C4 and C3 plus ^{125}I-C3. Classical complement pathway activation was determined by measuring binding of ^{125}I-C3 to JS3 after a 30-min incubation at 37°C. Specific binding of ^{125}I-C3 to JS3 occurred in the absence of added McAb 10, whether GPC1 or human C1 was used (Fig. 2). The extent of specific binding (molecules bound with C1423 minus molecules bound with C143) was 4,000 molecules C3/organism with GPC1 and 3,000 molecules/organism with human C1. When JS3 was presensitized with McAb 10, specific binding of C3 remained ~ 4,500 molecules of C3/organism, regardless of the source of C1. Irrelevant MAb neither blocked nor enhanced C3 binding (not shown). Because antibody-independent activation of C1 by JS3 was excluded earlier, the C3 deposition observed in the absence of McAb 10 can only be explained by the activation state of the C1 used in these experiments. GPC1 is available only in activated form and is capable of activating the remaining human classical pathway components. Human C1 zymogen subjected to the conditions of this assay (37°C for 30 min) spontaneously activates by the autocatalytic mechanism (37). In agreement with this model, when C1 (GPC1 or human C1) and C1-inhibitor (both at 5% normal serum concentrations) were added to gonococci before addition of C4, C2, and C3, deposition of C3 was completely abrogated (Fig. 3). Similar results were obtained when C1-inhibitor was added together with C4, C2, and C3 (not shown). Experiments were then done

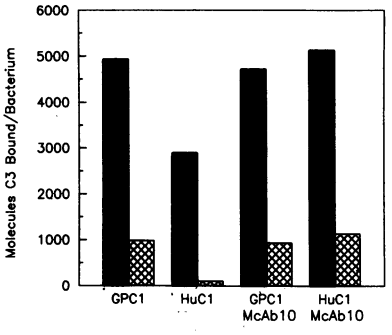

Figure 2. C3 binding to gonococci in the presence and absence of McAb 10. Strain JS3 (8×10^6) was presensitized with McAb 10 or buffer, incubated with GPC1 or human C1 at 30°C for 15 min, and then washed. Human C4, C2, and ^{125}I-C3 (*solid bars*) or C4 and ^{125}I-C3 (*hatched bars*) were mixed with the cells and incubated for 15 min at 37°C. Triplicate aliquots were removed, washed, and ^{125}I-C3 associated with the pellet was determined. C3 deposition occurred on JS3 in the presence or absence of McAb 10 using either GPC1 or human C1.

to determine if the C1 activation in the presence of McAb 10 was also prevented by C1-inhibitor. JS3 pretreated with McAb 10 was incubated with a mixture of human C1 and C1-inhibitor, followed by C4, C2, and C3. McAb 10 completely restored C1 activation and C3 binding by JS3 (Fig. 3).

Form of C3 bound to JS3. We analyzed the form of C3 deposited on JS3 with purified classical pathway components using SDS-PAGE and autoradiography. In the absence of methylamine (Fig. 4, *left*), C3-acceptor complexes migrating at 150,000 mol wt and larger were observed, as well as a large amount of α (120,000), α' (110,000) chain, and β chain (75,000) of C3b. The autoradiogram shown does not clearly demonstrate distinct α and α' bands, but both fragments are apparent in less heavily exposed autoradiograms. No such high molecular weight C3-acceptor complexes were noted in lanes containing samples lacking C2. This reinforced earlier results showing no specific binding of C3 unless all classical components were present. Upon treatment with methylamine (Fig. 4, *right*), C3 was released from acceptor molecules and migrated as C3b in the form of C3α' and C3β chains (110,000 and 75,000 mol wt, respectively). No bands corresponding to the 68,000-mol wt α'_1 fragment of iC3b or the 41,000-mol wt α'_2 fragment of C3dg were observed. The experiment shown was performed with GPC1, but was repeated several times using human C1 with the same outcome (not shown). These results indicate that a portion of C3 deposited with purified classical pathway components binds covalently as a portion of C3b to

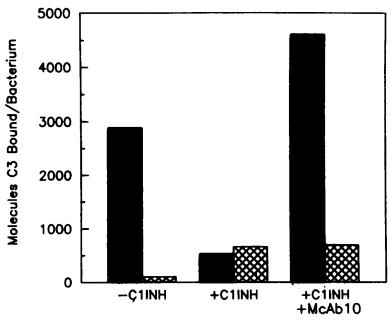

Figure 3. C1 activation in the presence of C1-inhibitor. JS3, with or without McAb 10 presensitization, was incubated at 30°C with a mixture of C1 and C1-inhibitor or C1 alone, washed, and then incubated at 37°C with C4, C2, and ^{125}I-C3 (*solid bars*) or C4 and ^{125}I-C3 (*hatched bars*). C1-inhibitor suppressed C3 deposition on JS3 to the level seen in the absence of C2. McAb 10 circumvented the effects of C1-inhibitor, and C3 deposition was restored.

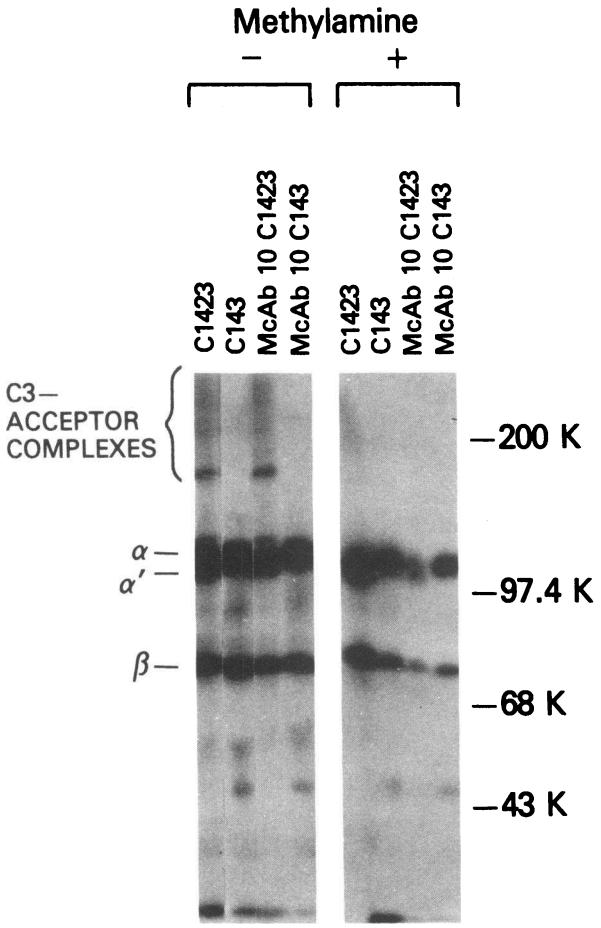

Figure 4. Autoradiogram of 7.5% SDS-PAGE demonstrating the form of C3 bound to JS3 and the oxyester nature of the bond. JS3, with or without presensitization with McAb 10, was incubated in purified C142 ^{125}I-C3 or C14 ^{125}I-C3 as described in Methods. Whole, washed organisms were analyzed by 7.5% SDS-PAGE autoradiography. (*Left*) High molecular weight C3-acceptor complexes at 150,000 mol wt and greater, along with free α, α', and β chain of C3b only in samples containing a complete set of the classical pathway components. (*Right*) Release by methylamine of C3b in the form of C3α' (110,000 mol wt) and C3β (75,000 mol wt). No smaller fragments not present in the pretreatment sample are apparent.

gonococcal constituents via an ester linkage, with no evidence for further cleavage.

Opsonization, phagocytosis, and killing of JS3. Gonococcal, strain JS3, preopsonized with various combinations of McAb 10 and classical complement pathway constituents, was tested in neutrophil phagocytosis and killing assays with PMN. Significant killing in comparison to buffer was noted for all combinations of opsonins tested (Table III); however, the combination of McAb 10 plus C1423 was significantly more bactericidal than either McAb 10 alone or McAb 10 plus C143. Although most experiments were performed using 20 μl of McAb 10 for presensitization, results were not substantially different when either 5 μl (34% survival) or 80 μl (25% survival) of McAb 10 were used in conjunction with C1423.

Discussion

This paper examines the control of complement activation on the surface of *N. gonorrhoeae.* In particular, our studies have focused on the interaction of the gonococcal surface with antibody (McAb 10) directed against the H.8 antigen and with classical pathway components. We have reported in this paper that McAb 10 has differential bactericidal activity for various strains of gonococci in adsorbed PNHS. We next showed antibody-independent C3b deposition on strain JS3 gonococci via the classical complement pathway. Although addition of C1-inhibitor blocked this activation, McAb 10 fully restored classical pathway activation and enhanced PMN phagocytosis and killing of gonococci. We have thus demonstrated, using a system of rigorously controlled, purified complement constituents, that antibody is necessary for classical pathway activation in the presence of C1-inhibitor by a clinical, serum-resistant isolate of *N. gonorrhoeae.*

Table III. Opsonization and Neutrophil Phagocytosis and Killing of Strain JS3 Gonococci

Opsonin	Amount of McAb 10	Survival at 60 min*
	μl	%
Buffer	0	82±16‡ (5)
C1423	0	43±3§ (3)
McAb 10	20	51±17 (2)
C1423 + McAb 10	5	34 (2)
	20	30±11§ (3)
	80	25 (2)
C143 + McAb 10	20	57±7 (2)

In parentheses, number of experiments performed.
* Incubation at 37°C in a candle-extinction jar.
‡ Mean±SD.
§ Significantly different from buffer by *t* test.

Complement activation is regulated in serum (and plasma) by C1-inhibitor. In the absence of this inhibitor, C1 can spontaneously autoactivate (reviewed in references 9 and 37–39). C1-inhibitor does not prevent C1 activation mediated by soluble immune complexes or by antibody-coated erythrocytes. A role for both antibody and for C4 in protecting activated C1 against inactivation by C1-inhibitor has been suggested (45).

A variety of substances and particles have been shown to activate C1 in the presence of C1-inhibitor, but in the absence of antibody (46–50). Various strains of gram-negative bacteria have been studied in this regard. Tenner et al. (6) showed that a strain of *E. coli* (J5) bearing rough LPS activated C1 directly in the presence and absence of C1-inhibitor. In contrast, the parent 0111B4 strain containing smooth LPS activated C1 in the absence, but not the presence, of C1-inhibitor. In this same study, Tenner provided indirect evidence that a two-site interaction of C1 with *E. coli* J5 was necessary for circumventing control of C1-inhibitor. Clas and Loos (9) made similar speculations based on their studies with purified LPS from *Salmonella* and from the capacity of C1 to interact directly with bacterial outer membrane proteins. Aubert (8) suggested that only a single-site interaction was required for C1 activation by *E. coli* strain D3m4. The structural requirements within LPS for direct C1 binding and classical pathway activation are also under study. Data of Vukajlovich and colleagues give the most lucid explanation of this problem. They suggest that D-manno-heptulose within *Salmonella* LPS abrogates classical

pathway activation (activating instead the alternative pathway) in serum (51, 52). Precise structural characterization of the LPS from *N. gonorrhoeae* has not been completed. The observation by Shafer et al. (4) that a "deep rough" mutant of *N. gonorrhoeae* lacking D-manno-heptose activated the classical pathway and was killed in agammaglobulinemic serum suggests that results with *N. gonorrhoeae* may be analogous to those with *Salmonella.*

Serum-resistant gonococci activate the classical pathway in non-immune or adsorbed human serum. Although C3 and C5b-9 are deposited on the bacterial outer membrane, killing does not occur (14, 28). Bactericidal rabbit polyclonal antibody alters the site of deposition or molecular configuration of C5b-9 bound to the gonococcal surface without increasing the amount of bound C5b-9 (28, 53). Similar results are reported for bactericidal human antibody for *N. gonorrhoeae* (53) as well as for some, but not all, murine MAb directed against the major gonococcal outer membrane protein PI (5). Such a change in form or site of binding of C5b-9 to some serum-resistant organisms is the most likely explanation for killing of serum-resistant JS3 by adsorbed PNHS supplemented with McAb 10.

The specific antibody, MAb10, enhanced neutrophil phagocytosis and killing of JS3. In the absence of serum, neutrophils avidly ingest piliated gonococci containing some but not all PII proteins (55, 56). In contrast, nonpiliated organisms lacking PII are not ingested in the absence of opsonization (55, 56). A rigorous dissection of the relative contribution of antibody and complement to phagocytosis of *N. gonorrhoeae* has not previously been attempted with purified opsonins. Our data suggest that both anti-H.8 IgG and C3b facilitate phagocytosis of JS3 by neutrophils, but that optimal phagocytosis is achieved with a combination of both ligands.

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