# IMMUNOGLOBULIN G ANTIBODIES DIRECTED AGAINST PROTEIN III BLOCK KILLING OF SERUM-RESISTANT *NEISSERIA GONORRHOEAE* BY IMMUNE SERUM

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Resistance to killing by normal human serum (NHS)[1] and complement is exhibited by most strains of *Neisseria gonorrhoeae* that cause disseminated gonococcal infection (DGI) (1). This phenotypic property of DGI strains is in contrast to that often observed in strains that cause locally invasive disease, e.g., pelvic inflammatory disease (PID), which frequently are highly sensitive to NHS (2). This feature may identify strains that are capable of promoting local inflammation (2). On the other hand, DGI strains usually cause no local symptoms (3). This may enable DGI strains to evade local defenses (4), penetrate local barriers, and invade the bloodstream. Serum-resistant organisms are also disproportionately represented in asymptomatic men (5) who do not develop disseminated infection.

The mechanisms of serum resistance and sensitivity are directly related to the efficiency of insertion of the membrane attack complex of complement (C5b-9) (6). Activation and deposition of individual complement components onto the surface of organisms either directly or via antibody are not sufficient, however, to ensure complete insertion of the membrane attack complex (MAC) (7, 8). Natural antibodies of the IgG class may subvert adequate insertion of the MAC. These have been described in human sera, and are termed blocking antibodies (7–9). These antibodies interfere with the efficient insertion of the MAC by as yet unknown mechanism(s); however, binding of these antibodies to particular antigenic targets on the surface of the gonococcus may divert the necessary localization of complement (C) away from bactericidal sites (7). Recent studies using murine monoclonal antibodies (mAb) have shown that antibody specific for a gonococcal surface protein, protein III, is able to block killing of gonococci by bactericidal antibody directed against a separate epitope (10). Protein III appears to be not only present in all strains of gonococci (11, 12), but biochem-

[1] *Abbreviations used in this paper:* DGI, disseminated gonococcal infection; LOS, lipooligosaccharide; MAC, membrane attack complex; NHS, normal human serum; PID, pelvic inflammatory disease.

ically and immunochemically identical (12, 13). In earlier studies, we have shown that IgG prepared from NHS with blocking activity contains antibody specificity for outer membrane proteins (9, 14). The studies reported here investigate the unique antigenic specificity of human blocking antibody and demonstrate the blocking action of purified IgG antibodies directed against gonococcal outer membrane protein III (PIII).

## Materials and Methods

### Strain

A single serum-resistant strain of *N. gonorrhoeae* isolated from the joint of a patient with disseminated gonococcal infection (DGI-1) was used in these studies. This strain resisted killing by 10 fresh NHS at serum concentrations of 66.7% and has a protein I (PI) of $M_r$ 36,200. It is in the IB-3 serovar group (15), kindly serotyped by Dr. Joan Knapp, Centers for Disease Control, Atlanta, GA, and auxotyped as Pro⁻ (16).

### Serums

Convalescent serum from the patient (DGI-1 immune serum), which expressed bactericidal activity against the infecting strain, was obtained 22 d after infection and used as the source of bactericidal antibody. Bactericidal antibody in this serum is directed against lipooligosaccharide (LOS) (17, 18) similar to other serums obtained from DGI patients in convalescence that sometimes express bactericidal activity against strains resistant to killing by NHS (1, 3). Blocking activity was identified using a previously described assay (9) in 4 U of citrated plasma (converted to serum by the addition of $CaCl_2$ to a final concentration of 0.15 M). Serum with blocking activity was recovered from this material and pooled. We also identified a second DGI convalescent serum (DGI-2 serum) that exhibited <50% killing against the DGI-1 test strain when used in the bactericidal assay at a concentration of 66.7%. Specific antibody was depleted from this serum to attempt the removal of blocking activity.

### Growth of Organisms

Transparent (Op⁻), nonpiliated (P⁻) phenotypes of DGI-1 gonococci were grown in mass culture. Preparation of outer membranes, isolated proteins, and LOS was accomplished using organisms that were grown on a solid clear media (19) for 16 h at 37°C in candle extinction jars or on liquid culture as previously described (20).

### Preparation of Antigens

Preparation of outer membranes was performed according to methods, which we have described previously (17), that yield membranes judged pure by their uniform density, $\rho°$ = 1.27 g/cm³ and absence of D-lactate dehydrogenase activity. Protein I was purified as described (21) and the purification of protein III was accomplished by methods described by Lytton and Blake (see preceding article [22]). These isolated proteins were assessed for purity by SDS-PAGE (23, 24) analysis and staining with silver nitrate (25, 26) and also for antigenicity in Western blotting (27) experiments that used specific murine mAb. mAb 2E6 exhibits specificity for protein III (13), and 3C8 recognizes a determinant present on protein I of the IB-3 serovar (15). LOS was prepared from whole organisms using a modification (28) of hot phenol/water extraction (29). This preparation and the individually purified proteins were used in ELISA (30) to validate the specificity of IgG antibodies that were immunopurified against specific outer membrane proteins.

### IgG Preparations

IgG subclasses 1, 2, and 3, but not 4 (31) were separated from the 4 U of pooled serum by anion-exchange chromatography using QAE-Sephadex A-50 (Pharmacia Fine Chemicals, Piscataway, NJ) gel equilibrated in a 0.048 M ethylene diamine, 0.073 M acetic acid buffer, pH 7.0 (32).

## Solid-phase Immunoabsorption

Individual proteins, protein III or I, were coupled to Sepharose 4B (Pharmacia Fine Chemicals) that was activated with cyanogen bromide and triethylamine, pH 7.0 (33, 34). This activation process favors the formation of cyanate esters that are predominantly responsible for covalent coupling of ligand to the resin (33, 34). Purified proteins III and I (1 mg/ml) were dialyzed extensively against 0.1 M NaHCO$_3$ buffer (pH 8.0) containing 0.5 M NaCl and 0.05% Zwittergent (Behring Diagnostics, Somerville, NJ). The solutions of proteins were added to the activated gels to obtain a 50% slurry and rotated end-over-end overnight at 4°C. After allowing the gels to settle, the supernatant was removed and assayed for protein (35). The remaining active groups were blocked by incubating the gels with 0.1 M ethanolamine-HCl 9 (pH 8.0) overnight at 4°C. The gels were finally equilibrated in 0.1 M Tris-HCl (pH 8.0). IgG preparations (0.5–1.5 ml) were equilibrated in PBS-Tween (0.05%) and applied to gel columns overnight (4°C) then washed with PBS-Tween. Additional samples of IgG were applied to the columns and the same procedure repeated for a total of three runs. After a final wash with PBS, columns were brought to room temperature, the PBS exchanged for 2 M KI, and after 1 h the antibodies were eluted from the columns with additional 2 M KI. The eluted preparations were exchanged into 0.15 M NaCl, concentrated, and used promptly in the immunologic assays. Control columns were also used, with mock-activated gels that were not reacted with gonococcal proteins, but were blocked with ethanolamine. Immunodepletion of protein III antibody from DGI-2 immune serum was also carried out after equilibration of the serum with PBS-Tween and $10^{-4}$ mM phenylmethylsulfonyl fluoride. Immune serum was passed over the protein III gel and control columns and used in the immunologic assays after Tween-20 was removed by absorption with beads made of a copolymer of styrene-divinyl benzene (Bio-Beads SM-Z; Bio-Rad Laboratories, Richmond, CA) (36).

## Characterization of Purified Antibodies and Immunodepleted Serum

*Western blots.* The antibody specificities of normal IgG and antibodies purified from normal IgG were examined by western blotting (27). In preliminary experiments the antigenicity of the isolated proteins III and I used to prepare the immunoabsorbants was also assessed using specific monoclonal antibodies: 2E6 for protein III (12) and 3G8 for protein I (15). After SDS-PAGE (23, 24) of antigen preparations, these were electroblotted to nitrocellulose paper (Bio-Rad Laboratories) in a solution of 20% methanol (vol/vol) containing 0.025 M Tris and 0.192 M glycine (pH 8.7). Transfer was carried out at constant current (250 mA) for 2 h at room temperature and the papers were washed in PBS with 0.05% Tween-20 (27). Antibody containing solutions were diluted in 1% BSA-PBS and 0.5 M NaCl. After the antibody was reacted with the nitrocellulose strips for 16 h at 4°C, specificity of binding was identified with alkaline phosphatase–conjugated goat anti–mouse IgG or goat anti–human IgG (Sigma Chemical Co., St. Louis, MO) and blue color was visualized after the addition of substrate containing nitroblue tetrazolium and indoxyl phosphate in dimethylformamide (37).

*Enzyme-linked immunosorbent assay (ELISA).* Direct binding activity of antibodies in serum, isolated IgG, and immunopurified or immunodepleted specimens were determined by using a kinetic ELISA (30, 38) that employed purified antigens as coated targets on microtiter wells. Quantitation of specific IgG antibodies was determined by comparing rate of substrate conversion (change in absorbance) with change in absorbance in separately performed IgG assays that used anti-IgG coated onto microtiter wells and known concentrations of an IgG standard (Behring Diagnostics) (39). Total IgG concentrations in specimens were also determined by using the identical assay performed for the IgG standards. Polystyrene microtiter plates with round U-bottomed wells (Dynatech Laboratories, Inc., Alexandria, VA) were coated (250 μl/well) at 37°C for 3 h with a solution of protein III (0.4 μg/ml) or protein I (2.5 μg/ml) in NaCO$_3$ buffer (pH 9.6) (30). LOS was coated (60 μg/ml) in barbital acetate buffer (pH 4.6) (40). These concentrations were predetermined to give optimal (highest) readings at the concentrations of specific antibody where kinetic ELISA were to be performed. The standard curves used to establish changes in optical densities vs. IgG concentration were developed in experiments that used wells

coated with affinity-purified goat anti–human IgG (Antibodies Inc., Davis, CA) at coating concentration of 100 $\mu$g/ml in NaCO$_3$ buffer (39). Plates coated with anti-IgG or antigen were shaken at 37°C for 3 h, after which they were stored overnight at 4°C and then washed four times for 10 min each, by inversion in PBS-Tween (28). Experiments were performed with (a) IgG antibodies immunopurified against proteins III and I, (b) whole DGI-2 serum, and (c) DGI-2 serum immunodepleted of protein III antibody. These preparations were diluted in PBS-Tween and dispensed into the wells coated with test antigens or anti–human IgG. Human IgG standards diluted in PBS-Tween were added at concentrations ranging from 20 to 200 ng/ml to the "standard curve" wells coated with antibody against human IgG. The remainder of the assay was performed as described previously (28) using anti–human IgG conjugated with alkaline phosphatase (Sigma Chemical Co.) and disodium $p$-nitrophenyl phosphate as substrate. Incubations with substrate were performed at 21°C and absorbance readings (405 nm) were taken at 5-min intervals for 30 min. A standard curve relating IgG concentration to rate of increase of absorbance was constructed and specimen values were determined from the curve. Controls used in these assays have been described previously (28).

Each ELISA experiment was performed in duplicate on three separate occasions and the standard error of the mean of three experiments was used as a measure of variance.

*Blocking assays.* Bactericidal conditions and controls that were used as a basis against which blockers were tested have been described previously (9). Test mixtures contained 0.075 ml of bactericidal serum and blockers, 0.025 ml of gonococci, and 0.05 ml of active complement. The following reagents were tested for their ability to block killing of the test strain by homologous convalescent serum from the infected patient (DGI-1): (a) IgG derived from normal human serum, and (b) normal IgG immunopurified against protein III or I. Complement was obtained from the serum of a 27-yr-old woman with acquired agammaglobulinemia (9).

IgG preparations or immunopurified IgG antibodies were diluted serially in 0.05 ml with 0.15 M saline and incubated for 15 min with the test dilution of the strain (0.025 ml) at 37°C. Thereafter, 0.025 ml of a dilution of convalescent DGI-1 serum that had produced an approximately ten-fold kill in the bactericidal assay, was added to the reaction mixture together with 0.05 ml of complement. The reaction mixtures were incubated at 37°C with continuous shaking in a water bath (New Brunswick Scientific Co., Inc., Edison, NJ). Viable colony counts were performed at 0 and 30 min by plating duplicate 0.025-ml samples of each reaction mixture and the percent killing taken as the reduction of CFU. Percent inhibition was calculated as the ratio of the differences in percent of killing between DGI immune serum alone and the test specimen with added blocker: [(percent kill, immune serum) − (percent kill, immune serum with blockers)]/[(percent kill, immune serum) − (percent kill, negative control)]. The mean percent inhibition and range was determined by performing each assay on two separate occasions.

Bactericidal experiments with the DGI-1 strain were also performed with whole DGI-2 serum and serum that had been immunodepleted of protein III antibody. DGI-2 serum that had been passed over mock columns was also tested as control. Concentrations of serum up to 80% were included in these reaction mixtures and the 33.33% concentration of complement was contributed by each of the serum preparations. Protein III–depleted serum and DGI-2 serum passed over control columns maintained 38% and 43%, respectively, of the original 120 CH$_{50}$ U/ml (41).

## Results

*Immunochemical Characterization of Isolated Proteins III and I Used for Immunopurification.* Isolated outer membranes and proteins III and I were examined by SDS-PAGE and western blotting to assess purity and antigenicity of isolated preparations. Fig. 1 demonstrates the gel patterns of isolated protein III and I (lanes *3* and *7*, respectively) compared with intact outer membranes (lane *2*). Recognition of isolated protein III by mAb 2E6 and protein I by mAb 3C8 (lanes *5* and *9*, respectively) indicated that antigenicity of the proteins was maintained
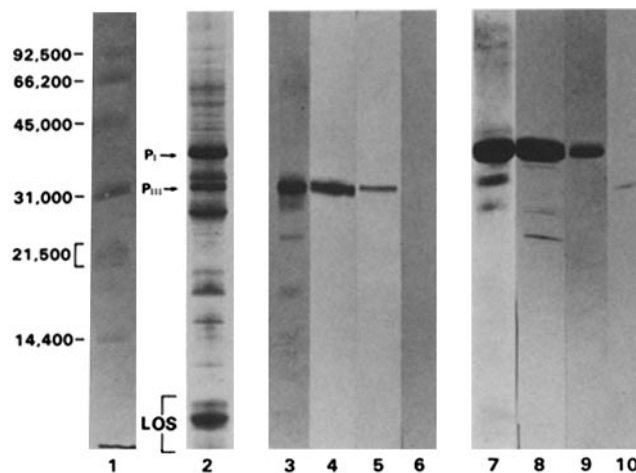
FIGURE 1. SDS-PAGE analysis of *N. gonorrhoeae* strain DGI-1 outer membrane proteins; immunochemical analysis by Western blots of (*a*) specific mAb against purified proteins III and I and (*b*) immunopurified human IgG antibodies against proteins III and I. Lane *1*, standards; lane *2*, SDS-PAGE of outer membranes of strain DGI-1; lane *3*, SDS-PAGE of purified protein III; lane *4*, human IgG immunopurified against protein III and reacted with protein III in Western blot; lane *5*, protein III-specific mAb 2E6 reacted with protein III in Western blot; lane *6*, protein I-specific mAb 3C8, reacted with protein III in Western blot; lane *7*, SDS-PAGE of purified protein I; lane *8*, human IgG immunopurified against protein I and reacted with protein I in Western blot; lane *9*, protein I-specific mAb 3C8, reacted with protein I in Western blot; lane *10*, protein I-specific mAb 2E6 reacted with protein I in Western blot.

through the purification process. The heterologous mAb did not recognize protein III material (lane *6*) although minimal contamination (<1% [20]) of the protein I preparation (not visualized with a sensitive silver stain [26], lane 7) was identified by mAb 2E6 (lane *10*). These isolated protein preparations served as effective immunoabsorbants when coupled to gels, as evidenced by successful rebinding to respective antigens of human IgG that had been immunopurified against protein III (lane *4*) and IgG immunopurified against protein I (lane *8*).

*Specificity of Immunopurified IgG.* Human IgG immunopurified against protein III and I antigens was assessed for specificity in western blotting experiments that employed the whole outer membrane as target antigen (Fig. 2). Although whole IgG was seen to bind to a number of proteins in addition to major gonococcal proteins III and I (also protein II [42], lane *3*), immunopurified antibody against protein III bound only to the protein III antigen (lane *4*). In separate experiments (not shown) this antibody also binds to reduction-modified protein III (11, 12) at a slightly higher $M_r$. Antibody purified against protein I was shown to bind principally at the location of protein I (36,200 $M_r$, lane *5*), however binding was also demonstrated at several additional locations; these locations corresponded in part to stained bands seen on the gel of protein I (Fig. 1, lane 7). This may also have included faint binding to a small amount of protein III present in this preparation. Antibody binding to LOS was not seen in western blots that used the whole IgG preparation, however, experimental conditions optimal for binding to outer membrane proteins were not favorable for IgG
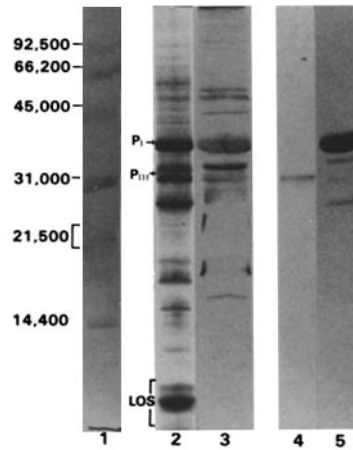
FIGURE 2.    SDS-PAGE analysis of *N. gonorrhoeae* strain DGI-1 outer membranes and Western blots of whole IgG and purified IgG antibodies reacted with outer membrane antigens. Lane *1*, standards; lane *2*, SDS-PAGE of outer membranes of strain DGI-1; lane *3*, human IgG reacted with outer membranes in Western blot; lane *4*, human IgG immunopurified against protein III and reacted with outer membranes in Western blots; lane *5*, human IgG immuno-purified against protein I and reacted with outer membranes in Western blots.

TABLE I

*Specific Antibody Activity\* in Serum-derived Fractions*

| Preparation | Target antigens[‡] | | |
|---|---|---|---|
| | Protein III | Protein I | LOS |
| IgG preparation | 2.08 ± 0.06 | 3.01 ± 1.0 | 0.70 ± 0.04 |
| Protein III eluent | 98 ± 7.2 | <0.06[§] | <0.06[§] |
| Protein I eluent | 0.07 ± 0.01 | 75.1 ± 10.1 | <0.06[§] |

\* (micrograms of measured antibody [IgG]) × $10^{-4}$/(micrograms IgG)
[‡] Antigens used in ELISA were prepared from DGI-1.
[§] ODs were less than negative controls.

binding to LOS antigens. This was evident when we measured IgG antibodies against LOS in the whole IgG preparation using ELISA (Table I).

Specific antibody activity against the two isolated proteins, in addition to LOS, was determined by ELISA (Table I). The specific activity of protein III antibody was enhanced 47-fold in the protein III eluent compared with the whole IgG preparation. The absence of measurable antibody against protein I in the protein III eluent confirmed results of western blotting. The specific activity of protein I antibody was enhanced 25-fold in the protein I eluent. 3.4% of protein III-specific activity remained in this preparation, possibly the result of a small amount of protein III that was present in the protein I preparation used for immunopur-ification. LOS antibody was not detected in either preparation, indicating >91% purity of these preparations from LOS antibody.

*Blocking Assays.*    Whole IgG and protein III and I antibodies immunopurified from IgG were tested for blocking activity. As previously reported (7, 9, 14), IgG blocks killing by immune human serum of gonococci that otherwise resist killing by NHS (Fig. 3). Purified protein III antibody effectively blocked killing
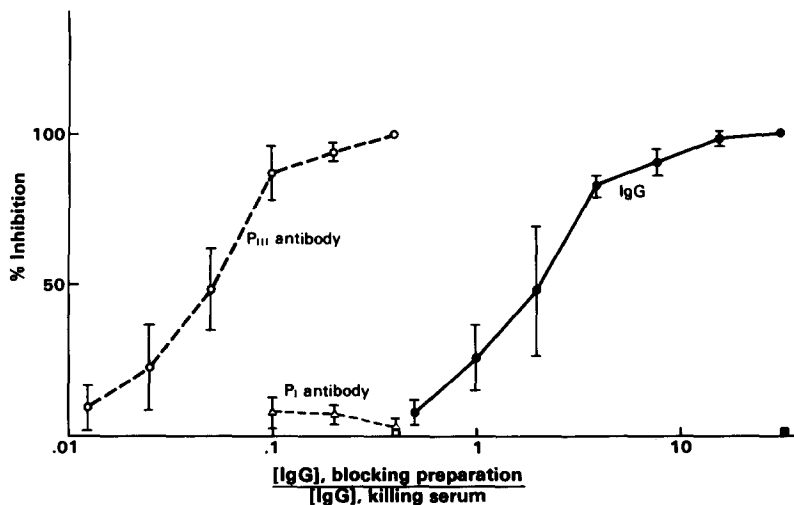
FIGURE 3. Inhibition of convalescent immune serum killing of *N. gonorrhoeae* strain DGI-1 by IgG isolated from NHS and by antibodies immunopurified against proteins III and I. Percent inhibition is expressed as a function of the ratio of IgG concentration in the blocking preparation divided by the IgG concentration in the killing serum. (□) Protein III (PIII) antibody heated to 65°C. (■) IgG heated to 65°C. *n* = two experiments ± range.

in a dose-related fashion. Purified protein I antibody displayed no blocking activity, and at the highest concentration used in these studies showed modest bactericidal ability in the absence of the killer immune serum (experiments not shown). Eluents recovered from control columns showed no blocking or bactericidal activity. Heating of IgG and antibody preparations to 65°C to destroy antibody function eliminated blocking activity (Fig. 3). Heating to 57°C for 30 min had no effect.

Protein III antibody requirements for blocking in whole IgG preparation was compared with that in the purified PIII antibody preparation. This comparison revealed that purified protein III antibody maintained blocking activity equivalent to protein III antibody in whole IgG (Fig. 4). These results suggest that blocking activity in whole IgG may be caused predominantly by the action of antibodies directed against PIII.

A second immune DGI serum (DGI-2) that had been chosen for study because it lacked killing activity at high concentration against the DGI-1 test strain was depleted of protein III antibody and tested in the bactericidal assay. Serum passed over the protein III immunoabsorbant column was depleted of 96% of protein III antibody while protein I and LOS antibody concentrations were maintained (Table II). Bactericidal assays were performed with protein III antibody depleted sera and its whole serum counterpart (Fig. 5). Whole serum demonstrated a bactericidal prozone (Neisser–Wechsberg phenomenon [43]) with emergence of killing as the serum was diluted. Selective removal of protein III antibody eliminated the prozone and unmasked killing activity at the highest concentration of absorbed serum used in the assay (Fig. 5). Whole serum passed over control columns maintained between 76 and 92% of blocking activity
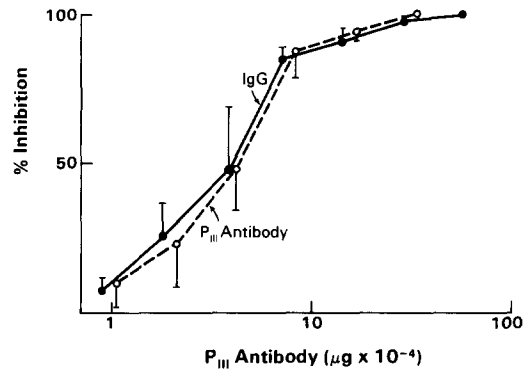
FIGURE 4.    Inhibition of convalescent immune serum killing of *N. gonorrhoeae* strain DGI-1 by whole IgG and purified protein III (PIII) antibodies. Percent inhibition is expressed as a function of the amount of PIII antibody, measured by kinetic ELISA, in blocking preparations that were employed in the assays. $n$ = two experiments ± range.

TABLE II

*Antibody Concentrations in DGI-2 Serum and DGI-2 Serum Immunodepleted of Protein III Antibody*

| Preparation | Target antigen* | | |
|---|---|---|---|
| | Protein III | Protein I | LOS |
| | $\mu g/ml$ | | |
| DGI-2 serum | 1.78 ± 0.15 | 12.21 ± 3.2 | 75.62 ± 7.2 |
| DGI-2 (immunodepleted of protein III antibody) | 0.073 ± 0.01 | 10.6 ± 5.4 | 71.3 ± 5.2 |

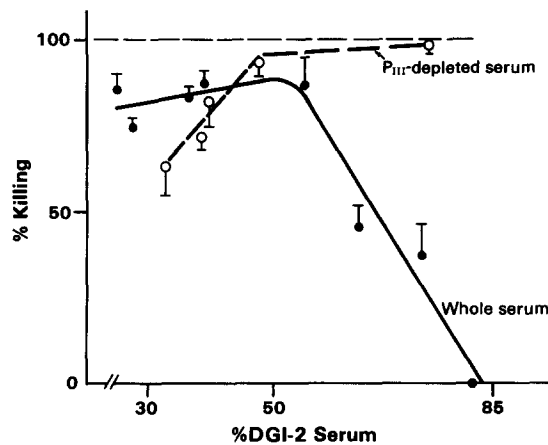* Antigens used in ELISA were prepared from the test strain (DGI-1).



FIGURE 5.    Killing action of DGI-2 serum and DGI-2 serum depleted of 96% of protein III (PIII) antibody, against *N. gonorrhoeae* strain DGI-1. Percent killing is expressed as a function of percent of serum in the bactericidal reaction mixture. $n$ = two experiments ± range.

present in unabsorbed serum, when tested at each of the concentrations indicated in Fig. 5 for whole serum.

## Discussion

We have shown in these studies that blocking IgG with specificity for a common gonococcal surface protein, protein III, contributes a major portion of blocking activity in normal and immune serums. Blocking IgG competes for binding sites on gonococcal surfaces with bactericidal antibody, resulting in effective prevention of complement-dependent killing (7). This contributes to an explanation of why certain strains of N. gonorrhoeae are resistant to killing by NHS. Immune and normal bactericidal antibodies against both serum-resistant and serum-sensitive strains of N. gonorrhoeae, respectively, are directed mainly against LOS (17, 44). Normal and convalescent serum may contain antibodies that recognize protein I; these antibodies may also be bactericidal (18) particularly when they are tested in the absence of blocking antibodies (unpublished observations). The intimate relationship of proteins III and I (13, 45, 46), which in turn appears to be tightly bound to LOS (47), indicates that binding sites for bactericidal and blocking antibodies are in close proximity. Based on estimates of the density distribution of LOS and protein I molecules on the surface of gonococci and their relationship to protein III, antibody binding to protein III might be expected to obscure certain binding sites present on either LOS or protein I.

Extensive serologic testing for the presence of protein III antibodies in NHS have not been performed, however many sera that have been tested contain IgG antibodies against this antigenically conserved surface protein (48, unpublished observations). In addition, the observation that the development of bactericidal activity in patients convalescing from DGI is often nonexistent or meager (3), despite high-titered antibody rises measured by indirect immunofluorescence (49) or ELISA (unpublished data), suggests that blocking antibodies may result from specific antigenic challenge. We have shown in these studies that selective protein III antibody depletion from DGI convalescent serum with unexpressed bactericidal activity restores killing activity to the serum.

Evidence of the specificity of binding of blocking antibody is also provided by our previous studies that have demonstrated equivalent blocking activity by F(ab')$_2$ fragments prepared from normal IgG (7, 9). We also found previously that low concentrations of F(ab')$_2$ blocking antibody effectively inhibited the binding of the protein III–specific mAb 2E6 to the strain used in the current studies (7). In a study that used a different serum-resistant strain, FA171, NHS was also shown to inhibit binding of mAb 2E6 (50). In those studies, most of the inhibition of mAb 2E6 binding was removed by absorption of NHS with purified protein I, which suggests that steric hindrance to nearby antibody combining sites also played a critical role in those competition binding studies. Using a third serum-resistant strain, R11, Joiner et al. (10) also demonstrated that mAb 2E6 could inhibit binding of a protein I mAb to the surface of this strain.

Serum bactericidal antibodies are associated with protection of the human host against invasive infection with N. meningitidis (51, 52) and Haemophilus influenzae (53). Currently, the role of complement-dependent serum bacterididal activity in the host defense against the three major categories of gonococcal infection—

uncomplicated, locally invasive (i.e., PID), and bacteremic infection is incompletely defined. Serum bactericidal activity directed against gonococci that cause uncomplicated local infection appears not to protect against this disease (54). Its absence in women with PID infected with gonococci, otherwise sensitive to NHS, may indicate susceptibility to locally invasive strains in these women (54). In addition, we have reported earlier that some DGI strains are killed by NHS, yet patients infected with these strains lack bactericidal activity at the time they are infected (3). We have also observed variations in resistance or sensitivity to the bactericidal action of NHS of strains that cause different types of invasive disease and this may prove to be an important factor that enables strains to produce varying patterns of inflammation resulting in the distinct manifestations of gonococcal infection (2, 3).

The complex interaction between the surface antigens of gonococci and the host may reflect both the inflammatory potential and the sensitivity to serum of *N. gonorrhoeae*. The contribution of blocking antibodies to the varying manifestations of gonococcal infection has not been clarified, but these antibodies have also been described with infections caused by other bacteria. In the first written account of serum blocking activity, Neisser and Wechsberg (43) described a wide range of gram-negative organisms where the killing action of normal fresh animal sera was blocked by immune sera taken from animals that had been immunized with whole bacteria. In 1943, Thomas and Dingle (55) described the loss of natural bactericidal activity in normal rabbits after passive intravenous immunization with antimeningococcal horse serum. This resulted in longer periods of bacteremia after the intravenous injection of meningococci. Sera taken from patients early in the course of meningococcal disease may have lytic activity against the infecting strain that is unmasked only when the sera have been freed of IgA (56). Convalescent sera from patients infected with the meningogoccus may show diminished bactericidal activity against the infecting strain and may also inhibit killing of meningococci by sera from normal humans (57). Similar inhibition has also been demonstrated in the sera of patients chronically infected with common enteric Gram-negative bacilli (58–60) and in the IgA fraction of sera taken from patients with chronic brucellosis (61).

The mechanisms of action of the blocking antibody for *N. gonorrhoeae* has not previously been known. In addition to displacing bactericidal antibody from gonococcal surfaces, it has been speculated that, like blocking antibody for *N. meningitidis* and *Brucella abortus*, which are of the IgA isotype and therefore unable to activate complement effectively, blocking IgG for *N. gonorrhoeae* and for other Gram-negative organisms (58–60) may be predominantly those isotypes of IgG that activate complement poorly. Blocking IgG, used in our studies, contained exclusively complement-fixing subclasses (IgG4 was excluded), and in separate studies it has been shown that organisms opsonized with blocking IgG increased consumption and deposition of the third (C3) and ninth (C9) components of complement (7). While ~25% of C3 deposited onto *N. gonorrhoeae* in the presence of blocking antibody is covalently bound to the antibody molecule, C3 deposition is also redirected to new sites on the gonococcal surface that do not result in serum killing (7).

Although our studies have shown that human sera contain protein III–specific

antibodies that are predominantly responsible for blocking activity, nonetheless it is possible that the nature of the antibody itself or the location of the epitope on the organism recognized by the antibody, in addition to the antigenic specificity, may dictate whether killing or blocking will supervene. Such an assumption is based on several observations: (a) different ratios of protein III–blocking antibody to bactericidal antibody are required in individual sera to achieve constant blocking (unpublished observations); (b) different mAb, all directed at a single protein I, can vary in bactericidal activity for N. gonorrhoeae, and indeed, nonkilling mAb against protein I can effectively block killing antibodies that recognize different epitopes on this protein (13); (c) IgA blocking antibody, which is specific for LOS, can sometimes block IgG anti-LOS killing of serum-resistant gonococci (62); and (d) protein III mAb 2E6 is bactericidal for some strains of N. gonorrhoeae at high concentrations (K. A. Joiner and J. Swanson, unpublished observations; P. A. Rice, unpublished observations). In humans, however, protein III antibody competes for binding with bactericidal antibody, leads to deposition of nonbactericidal C5b-9, and can prevent killing of gonococci by bactericidal antibody.

## Summary

*Neisseria gonorrhoeae* that resist complement-dependent killing by normal human serum (NHS) are sometimes killed by immune convalescent serum from patients recovering from disseminated gonococcal infection (DGI). In these studies, killing by immune serum was prevented or blocked by IgG isolated from NHS. Purified human IgG antibodies directed against gonococcal protein III, an antigenically conserved outer membrane protein, contained most of the blocking activity in IgG. Antibodies specific for gonococcal porin (protein I), the major outer membrane protein, displayed no blocking function.

In separate experiments, immune convalescent DGI serum which did not exhibit bactericidal activity was restored to killing by selective depletion of protein III antibodies by immunoabsorption. These studies indicate that protein III antibodies in normal and immune human serum play a role in serum resistance of *N. gonorrhoeae*.

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