Human Seminal Plasma Inhibition of Antibody Complement-mediated Killing and Opsonization of *Neisseria gonorrhoeae* and Other Gram-Negative Organisms

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ABSTRACT Seminal plasma diluted 1:5–1:1,000 gave marked inhibition of serum antibody complement-mediated bactericidal and opsonic effects against *Neisseria gonorrhoeae* and other gram-negative organisms. Serum that was bactericidal at a dilution of 1:5,120 was not bactericidal at a dilution of 1:10 when seminal plasma was added. Bactericidal action of immune human or rabbit sera, or purified immunoglobulin (Ig)G or IgM plus complement for six strains of *N. gonorrhoeae*, serogroups A, B, C, and Y of *Neisseria meningitidis*, *Escherichia coli* and other gram-negative rods was inhibited by seminal plasma. Using C8- or C7-deficient sera as antibody and complement sources, opsonization, phagocytosis, and killing of *N. gonorrhoeae* and *E. coli* 014-K7 were inhibited by seminal plasma. Opsonization, phagocytosis, and killing of *Staphylococcus aureus* 502A was not inhibited. For the gram-negative organisms, the early phase of the opsonization process, probably complement activation, appeared to be inhibited rather than the ingestion or polymorphonuclear leukocyte killing steps; addition of seminal plasma yielded a significant reduction in the percentage of polymorphonuclear cells with associated bacteria. Seminal plasma did not prevent attachment of IgG, IgM, or IgA antibodies to gonococci. It reduced serum hemolytic whole complement activity by 25%. The seminal plasma inhibitor was of low molecular

weight and was stable at 56°C for 30 min, but inhibitory activity was lost after heating to 100°C for 10 min. It is likely that the inhibitory factor(s) is a low-molecular weight protease or protease inhibitor. Seminal plasma probably has an important role in inhibition of complement and antibody functions in the genital tract. It may enhance pathogenesis of agents of sexually transmitted diseases.

INTRODUCTION

Highly effective biological mechanisms must be present to protect antigenically foreign spermatozoa from the cellular and humoral immune mechanisms potentially active in the female genital tract. These immunological mechanisms probably evolved to protect sensitive steps in reproduction, but also could function to promote the survival and enhance the virulence of *Neisseria gonorrhoeae* and other agents that cause sexually transmitted diseases.

Seminal plasma from humans, mice, and cattle contains a variety of poorly characterized, but potent, substances that inhibit in vitro reactions of lymphocytes from homologous or heterologous species (1–4). Seminal plasma inhibits pokeweed mitogen, phytohemagglutinin, concanavalin A, tetanus toxoid, and *Candida* antigen-induced lymphocyte activation. Seminal plasma also inhibits allogeneic cell stimulation in the mixed lymphocyte reaction as well as the generation of cytotoxic T cells against tumor antigens. Mouse anti-sheep erythrocytes in vitro plaque responses to T cell-dependent and -independent antigens are inhibited. This broadly active seminal plasma lymphocyte inhibitory activity elutes in a high-molecular weight peak from a Sephadex G-150 column.

The purpose of this report is to present observations on seminal plasma inhibition of antibody and complement-mediated bactericidal and opsonic systems.

METHODS

Human specimens used in this study were obtained under the guidelines of the University of California San Francisco, Committee on Human Research.

Seminal plasma. The seminal plasma specimens were from unidentified donors. The specimens had been collected for routine laboratory evaluations of infertility/fertility, for postvasectomy evaluations, and from normal volunteers. The specimens were allowed to liquify at room temperature, centrifuged to remove sperm, and frozen at $-13°C$. Specimens obtained in this manner were thawed, recentrifuged (5,000 g, 10 min) and pooled in volumes of 5-20 ml. Aliquots were frozen and stored at $-70°C$. Before use, the specimens were thawed, recentrifuged, and filtered through a 0.45-μm pore size filter (Millipore Corp., Bedford, Mass.). The centrifugation steps removed almost all sperm and no sperm were visible by light microscopy after the filtration step. A few specimens were handled individually in the same manner as the pooled specimens for evaluation of specimen-to-specimen variation of activity. Additionally, one large volume of pooled seminal plasma was kindly provided by Dr. Chung Lee and associates, Northwestern University School of Medicine.

Sera. Human sera used in these studies were obtained from blood drawn by venipuncture. The blood was clotted, centrifuged, and the sera were frozen in small aliquots at $-70°C$ within 90 min. Normal sera were obtained from healthy young adults. Complement-donor sera (Cd)[1] were from five patients with marked hypogammaglobulinemia; these sera were used individually as sources of complement. Gonorrhea patient sera were collected from study patients as described (5, 6). Immune human sera to *Neisseria meningitidis* sero-groups A, B, C, and Y were kindly provided by Dr. J. McCloud Griffiss, Walter Reed Army Institute for Research. Rabbit sera immune to *N. meningitidis* were kindly supplied by Dr. Neylan Vedros, University of California, Berkeley. Serum deficient in the eighth component of complement (C8) was obtained from a patient previously described in detail (7). Serum deficient in the seventh component of complement (C7) was kindly provided by Dr. Terrence J. Lee, University of North Carolina (8).

Bacterial isolates. The *N. gonorrhoeae* isolates studied were from the authors' culture collection (5, 6). *N. meningitidis* of sero-groups A, B, C, and Y were supplied by Dr. Vedros. Serum-sensitive strains of *Escherichia coli*, *Citrobacter*, *Klebsiella*, *Providencia rettgeri*, and *Serratia* were kindly supplied by Dr. Lowell S. Young, University of California, Los Angeles. *Staphylococcus aureus* strain 502A was provided by Dr. Arthur White, Indiana University School of Medicine.

The *N. gonorrhoeae* and other bacteria were maintained frozen at $-70°C$. For use in bactericidal and opsonic assays, the organisms were thawed and subcultured on GCI agar (GC agar base, BBL Microbiology Systems, Becton, Dickinson & Co., Cockeysville, Md.) with Iso-VitaleX (BBL Microbiology Systems) and incubated at 36.5°C in a 5% CO_2 incubator. The *N. gonorrhoeae* were piliated colony phenotypes (9) and were of intermediate opacity-transparency.

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[1] *Abbreviations used in this paper*: Cd, complement donor sera; cfu, colony-forming units; CH_{50}, hemolytic whole complement.

Bactericidal assays. The medium used for the bactericidal assays was: medium 199 (obtained 10 times concentrated, Microbiological Associates, Walkersville, Md.); 0.025 M Hepes buffer, pH 7.4 (Microbiological Associates); 0.5% bovine serum albumin (Miles Laboratories, Inc., Elkhart, Ind.) and 20% Cd as described (5-7). Heat-inactivated (56°C, 30 min) test serum was serially diluted from 1:10 to $\geq$1:5,120 for analysis of titer of bactericidal activity. The *Neisseria* species were grown 16-18 h on GCI agar and dispersed in medium 199 to make the inocula. The gram-negative rods were grown to log phase in Mueller-Hinton broth, sedimented by centrifugation and diluted in medium 199 to make the inocula.

The bactericidal assays for *N. gonorrhoeae* and *N. meningitidis* were done using a replicator device as described (6) with an inoculum of $\sim 6 \times 10^3$ colony forming units (cfu)/ml in a total vol of 0.25 ml. Bactericidal assays for gram-negative rods were done using an inoculum of about 1×10^4 cfu/ml in snap-cap plastic tubes with a total vol of 1.0 ml; rapid and large amounts of growth of the gram-negative rods precluded counting of colonies when bactericidal assays on these organisms were done with the replicator.

Serum immunoglobulins were fractionated by the method of Griffiss (10) and the purified immunoglobulin preparations were used in bactericidal assays.

Opsonization, phagocytosis, and killing assays. The opsonization experiments were done with modifications of the techniques of Klebanoff (11, 12). To prepare leukocytes from peripheral blood, 10-ml vol of whole blood from normal donors were mixed with 10-ml vol of 2% dextran T 250 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) in physiological saline, pH 7.4. After sedimentation of the erythrocytes for 20 min, the supernates were collected and the leukocytes were gently pelleted (150 g, 10 min). The leukocytes were washed with 0.87% ammonium chloride to lyse the remaining erythrocytes, recentrifuged, mixed with calcium-free Krebs-Ringer phosphate buffer, and the percent and absolute number of polymorphonuclear cells were enumerated in a hemocytometer.

The studies of opsonization of *N. gonorrhoeae* and *E. coli* 014-K7 used C8- or C7-deficient sera as complement sources because sera with intact complement systems were bactericidal. The C8- and C7-deficient sera had normal opsonic activities (7, 8). Normal sera or C8 deficient serum were used to opsonize *S. aureus*.

To make inocula for the opsonization studies, the *Neisseria* species were grown and prepared in the same manner as for the bactericidal assays. The *E. coli* and *S. aureus* were grown to log phase in Mueller-Hinton broth, washed with water, and diluted in medium 199. In the opsonization assays, the inocula were $2-4 \times 10^7$ cfu/ml and the numbers of polymorphonuclear cells were $1-2 \times 10^7$/ml.

Phagocytic indices. To determine the percentage of polymorphonuclear cells with associated bacteria, similar mixtures of bacteria, sera, seminal plasma, and polymorphonuclear cells were used as for the opsonization-phagocytosis assays. The reaction mixtures were incubated for 30 min and the suspensions were spread on glass slides, ethanol fixed and stained with Wright-Giemsa stain. Slides were read by an observer who did not know the composition of the reaction mixtures. Fields were randomly selected (100 magnification) and 100 or 200 consecutive polymorphonuclear cells were evaluated for cell-associated bacteria. Cells with associated bacteria were counted as positive when any surface of the polymorphonuclear cell was in proximity to an organism. The phagocytic index was defined as the percentage of polymorphonuclear cells with associated bacteria.

Seminal plasma and attachment of immunoglobulins and the third component of complement (C3) to gonococci. To

test the effect of seminal plasma on immunoglobulin binding, C8-deficient serum was serially diluted 1:2–1:1,024 and reacted with 10^7 cfu of *N. gonorrhoeae* strain g867 or *E. coli* 014-K7, in buffered medium for 30 min at 37°C. The organisms were washed once, resuspended in phosphate-buffered saline, and 25 μl was dropped on a glass slide, air dried, and gently heat fixed. The spot was overlayed with 25 μl of previously titered fluorescein-conjugated goat antibody to heavy chains of human IgG, IgM, or IgA (N. L. Cappel Laboratories, Inc., Cochranville, Pa.). After incubation for 30 min at 37°C, the slides were washed, air dried, and scored for degree of fluorescence as follows: no staining or 1+ were considered negative fluorescence; 2+ to 4+ were considered positive. To test the influence of seminal plasma on immunoglobulin binding to organisms, 10% seminal plasma was preincubated with the organisms for 20 min before addition of the C8-deficient serum.

A similar set of experiments was done using radiolabeled anti-human goat antibodies. Iodination of the IgG fraction of goat anti-human IgG, IgM, and IgA heavy chain specific antibodies (N. L. Cappel Laboratories, Inc.) was done using described techniques (13, 14). Briefly, glass tubes were coated with 200 μl of 2% IODO-GEN (Pierce Chemical Co., Rockford, Il.) in methylene chloride. The tubes were dried with nitrogen gas. In a separate tube, the immunoglobulin (6×10^{-10} mol) was mixed with 20 μl of 17 Ci/mg^{125}I (New England Nuclear, Boston, Mass.) and 3.5 μl of 0.1% KI. The volume was adjusted to 100 μl with 0.125 M Na borate-0.075 M NaCl, pH 8.2. The reaction was initiated by transferring the immunoglobulin solution to the IODO-GEN-coated tube and allowed to proceed at 4°C for 5 min with gentle agitation. The reaction was terminated by chromatographing the mixture on a Sephadex G-100 (Pharmacia Fine Chemicals, Inc. Piscataway, N. J.) column equilibrated with 1% bovine serum albumin in phosphate-buffered saline.

The ^{125}I-labeled antibodies were used to test the attachment of the human immunoglobulins (from C8-deficient serum or normal serum) using a technique similar to that used for the fluorescein-labeled anti-immunoglobulin experiments. The sera were diluted from 1:10 to 1:10,000 and reacted with the bacteria. After incubation and washing to remove excess serum, unlabeled goat antiserum containing the ^{125}I-labeled antibody was added to each pellet and incubated at room temperature for 30 min. The pellets were washed four times and the ^{125}I activity in the bacterial pellets was determined.

The C8-deficient serum was used to test for C3 attachment to *N. gonorrhoeae* and *E. coli*. Fluorescein conjugated anti-C3 antibody (N. L. Cappel Laboratories, Inc.) was used to stain for C3. Fluorescence was graded the same as for the immunoglobulin attachment.

Chemiluminescence assays. Polymorphonuclear cell chemiluminescence was assayed by the method of Stevens et al. (15). Briefly, human polymorphonuclear cells were prepared as for the phagocytosis experiments. For preopsonization, *E. coli* were incubated for 30 min at 37°C in 50% fresh autologous normal serum, pelleted, and resuspended in the Krebs-Ringer phosphate buffer. Luminol (Sigma Chemical Co., St. Louis, Mo.) was kept in a stock 0.1-M solution in dimethyl sulfoxide and diluted for use in Krebs-Ringer phosphate buffer. The chemiluminescence was measured in a Searle liquid scintillation counter (Searle Radiographics Inc., Des Plaines, Ill.) with one photomultiplier tube disconnected; vials were counted for 30 s at 10-min intervals. The components of the chemiluminescence assays were mixed together in dark adapted liquid scintillation vials just before the initial determinations of counts per minute. The 2-ml chemiluminescence assays contained the following components, singly or in

various combinations: 10^5 polymorphonuclear cells; 5×10^6 cfu of *E. coli*; nM phorbal myristic acid (Sigma Chemical Co.); 0.1 μM Luminol; 1 or 10% seminal plasma; and Krebs-Ringer phosphate buffer.

Complement assays. Hemolytic whole complement (CH_{50}) assays were performed in three separate laboratories using described techniques (16–18). In each of the laboratories, sheep erythrocytes were obtained from the Colorado Serum Co., Denver, Colo., and preserved in modified Alsever's solution. Rabbit antisera (hemolysin) to sheep erythrocytes was purchased from Gibco Laboratories, Grand Island Biological Company, Grand Island, N. Y. or from BBL Microbiology Systems.

Studies of the seminal plasma characteristics. Crude seminal plasma was chromatographed on a 2.6 ×70-cm Sephadex G-25 (Pharmacia Fine Chemicals, Inc.) column equilibrated with 0.5% NH_4HCO_3. After spectrophotometrically measuring absorption at 280 nm, protein fractions were pooled. Pools were lyophilized for 24–72 h to concentrate the samples; this procedure removed essentially all of the NH_4HCO_3 (NH_3 and CO_2) as monitored by change in pH to neutrality. Dialysis of crude seminal plasma and of pooled elution fractions was done in standard cellulose dialysis tubing for 24 h against 0.5% NH_4HCO_3. The dialyzed materials and the dialysates were concentrated by lyophilization; the concentrated materials were screened for inhibitory activity at dilutions of 1:2.5.

Seminal plasma diluted 1:2 was treated with trypsin (Sigma Chemical Co.) in a final concentration of 1.25–2.5 mg/ml of seminal plasma. The mixture was incubated for 4 h at room temperature or at 37°C. Trypsin activity was stopped with soybean trypsin inhibitor used at the same concentrations as trypsin. These preparations were tested for effects of seminal plasma bactericidal inhibitory activity. Similar experiments were done using the soybean trypsin inhibitor alone, chymotrypsin (Sigma Chemical Co.) at 1.25–2.5 mg/ml of seminal plasma, and protease type IV (Sigma Chemical Co.) at the same concentrations.

Purified spermine and spermidine used in bactericidal assays (Sigma Chemical Co.) were supplied by Dr. Laurence Marton, University of California, San Francisco. The spermine and spermidine were used in concentrations approximating those in seminal plasma.

Protein determinations on seminal plasma were done by the method of Lowry et al. (19).

Statistical analysis of bactericidal data. A decrease of >40% of the cfu per milliliter in a bactericidal assay was significant compared with that in a sample obtained at zero time. The 40% decrease was more than the 95% confidence limits ($P < 0.05$) determined by comparing zero time and 40-min samples from more than 75 paired heated serum controls (5, 6). Heated sera were not bactericidal. In experiments designed to measure the titers of active materials, there was very rarely a difference of more than one dilution in the bactericidal titers determined on different days. We found no differences of more than two dilutions. Each experiment was performed at least twice and at the most four to six times with comparable results each time.

Student's *t* test was used to test significance of differences in phagocytic indices.

RESULTS

Seminal plasma inhibition of serum killing of N. gonorrhoeae. Seminal plasma diluted 1:5 markedly inhibited the bactericidal action of serum on *N. gonorrhoeae.* As demonstrated in Fig. 1, with the addition of
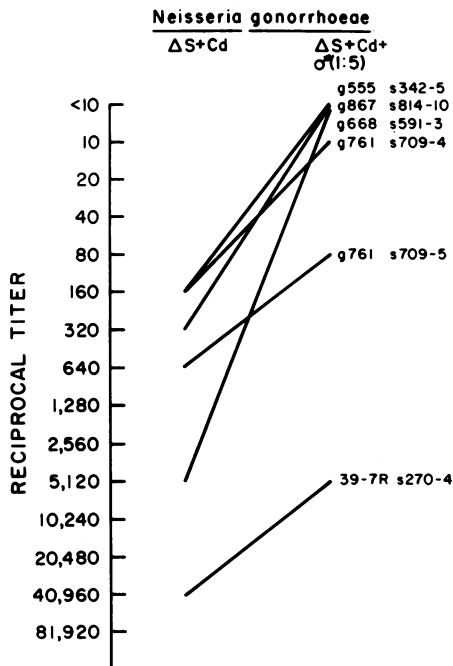
Neisseria gonorrhoeae

FIGURE 1 Reciprocal bactericidal titers of heat-inactivated (56°C, 30 min) human sera (ΔS) plus Cd are shown on the left. The bactericidal titer of each of the ΔS plus Cd combinations was greatly decreased by addition of seminal plasma (♂) diluted 1:5. The specific *N. gonorrhoeae* isolates (g numbers) and patient sera (s numbers) are indicated on the right.

complement-donor serum, *N. gonorrhoeae* strain 668 was killed by heated serum 591-3 diluted 1:5,120. Addition of seminal plasma diluted 1:5 inhibited the bactericidal action of serum 591-3 to a dilution of <1:10. There also was marked inhibition by seminal plasma of bactericidal effect with other combinations of sera and *N. gonorrhoeae* isolates (Fig. 1). Seminal plasma, alone or with Cd or heated serum, did not affect gonococcal viability in any of these experiments and the Cd or heated serum alone were not bactericidal. The sequence of preincubation of seminal plasma with each of the reaction components did not affect the inhibitory action of seminal plasma or bacterial killing. Pretreatment of *N. gonorrhoeae* with seminal plasma followed by washing of the bacteria did not affect the bactericidal action of serum. However, centrifugation during the washing of the *N. gonorrhoeae* did yield a loss of gonococcal viability of up to 90%, as reported (20).

Serum 591-3 (bactericidal titer for gonococcus g668, 1:5,120) was diluted 1:100 or 1:1,000 and used to test for variation in individual seminal plasma specimen inhibitory activities. With S591-3 diluted 1:100 plus Cd the six seminal specimens were inhibitory in titers of 1:40 to 1:160 (Table I). After dilution of S591-3 to 1:1,000, the seminal plasma inhibitory titers were 1:640 to ≥1:5,120.

TABLE I
Seminal Plasma Inhibitory Titers for Bactericidal Action of Serum S591-3 (Bactericidal Titer 1:5,120) against N. gonorrhoeae Strain g668

	Reciprocal ♂ inhibitory titer	
♂ specimen No.	S591-3 1:100	S591-3 1:1,000
1	40	2,560
2	160	≥5,120
3	160	640
4	80	≥5,120
5	80	640
12	80	—

Heated (56°C, 30 min) S591-3 was diluted 1:100 or 1:1,000, a standard amount of complement was added, and the inhibitory titers of seminal plasma (♂) specimens were determined.

Seminal plasma inhibition of serum killing of other bacteria. The specificity of seminal plasma inhibition of serum bactericidal action was tested with other species of bacteria. Bactericidal action of heat-inactivated serogroup specific rabbit immune serum plus human complement, or heat-inactivated human immune serum plus complement, for *N. meningitidis* also was markedly inhibited by seminal plasma (Fig. 2). The decomplemented sera and Cd alone or with seminal
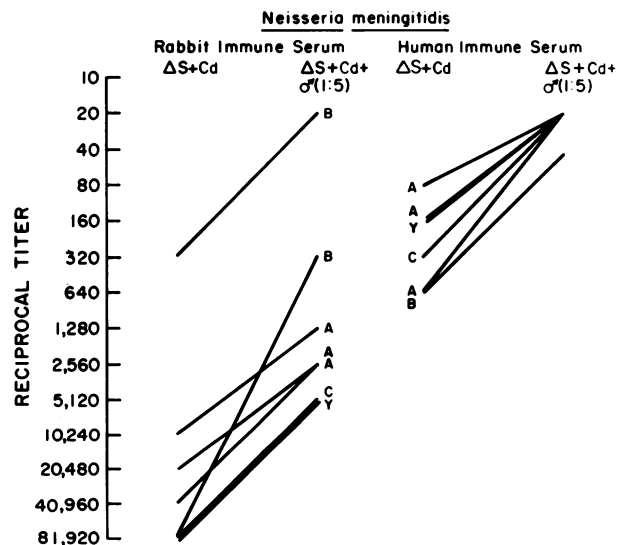

FIGURE 2 Reciprocal *N. meningitidis* bactericidal titers of heat-inactivated (56°C, 30 min) immune sera (ΔS) from rabbits or humans with added Cd. For both rabbit and human sera, the bactericidal activity was greatly reduced by addition of seminal plasma (♂) diluted 1:5. The serogroups (A, B, C, and Y) of the *N. meningitidis* are indicated. The immune sera were serogroup specific.

plasma were not bactericidal. Seminal plasma also inhibited the bactericidal action of 10% fresh normal human serum with intact complement for the highly serum susceptible strain E. coli 014-K7 (Fig. 3). Seminal plasma inhibition of the serum bactericidal effect on other gram-negative rods was less pronounced (Table II) and appeared to be related to the susceptibility of the organisms to 10% normal human serum.

Seminal plasma inhibition of opsonization, phagocytosis and killing of gonococci and E. coli. N. gonorrhoeae and E. coli opsonized by C8-deficient serum were phagocytosed and killed by polymorphonuclear cells. Seminal plasma inhibited one or more components of the sequence as measured by the endpoint of bacterial viability (Figs. 4 and 5). Nearly identical results were found when C7-deficient serum was used as the opsonin and N. gonorrhoeae was the test organism. In five experiments, seminal plasma had no apparent effect on normal serum or C8-deficient serum opsonization and polymorphonuclear cell phagocytosis and killing of S. auerus 502A.

Phagocytic indices. Seminal plasma appeared to inhibit the association of N. gonorrhoeae or E. coli with

TABLE II
Inhibition of the Bactericidal Action of Normal Fresh Human Serum against Enterobacteriaceae by Seminal Plasma Diluted 1:10

Organism	0 min	40 min	
	S	S + ♂	S
		cfu	
E. coli 014-K7	1.5×10^4	3.0×10^3	2.0×10^1
E. coli 3933	2.1×10^4	2.1×10^3	8.0×10^1
Citrobacter 3948	1.6×10^4	1.0×10^3	8.0×10^1
Klebsiella 3717	1.1×10^4	1.5×10^3	5.9×10^2
P. rettgeri 3874	1.2×10^4	9.1×10^3	3.5×10^3
Serratia 3996	1.4×10^4	3.1×10^3	3.0×10^3

S, human serum; ♂, seminal plasma.

polymorphonuclear cells as indicated by the phagocytic indices. In each of the experiments using C8-deficient serum as the complement source, ~50% of the polymorphonuclear cells had associated bacteria. When seminal plasma was added, the phagocytic indices were significantly decreased (Table III). The low percentage also was seen with polymorphonuclear cells alone, heated serum, and seminal plasma con-
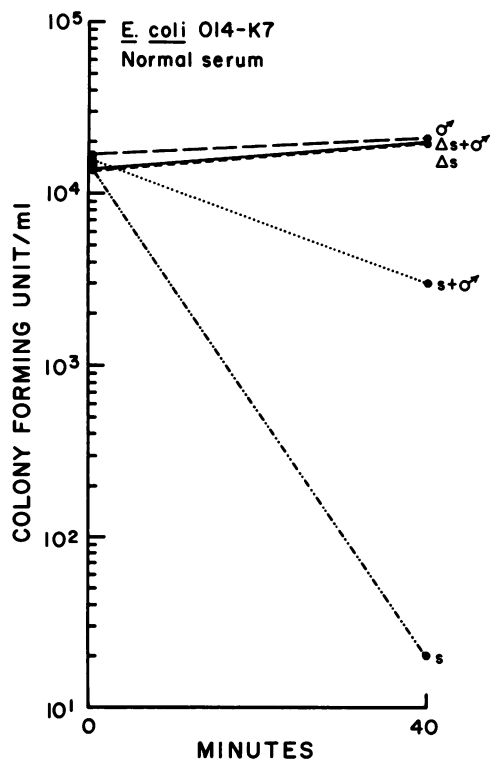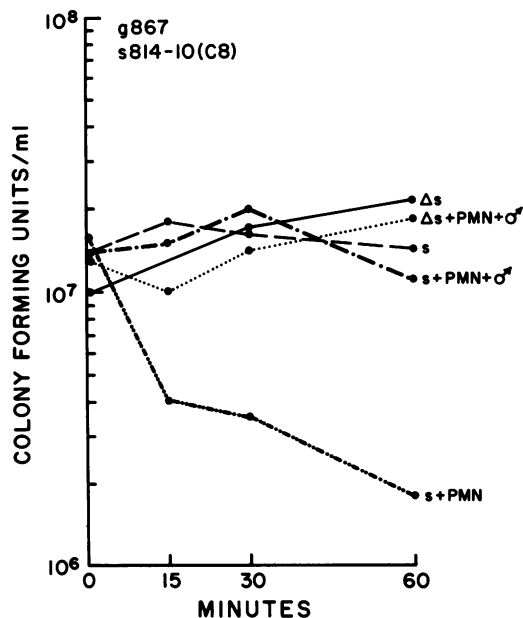
FIGURE 3   Bactericidal activity of normal human serum (s) for E. coli 014-K7. A decrease in cfu of >2 $\log_{10}$ in 40 min was noted. Seminal plasma (♂) diluted 1:5 inhibited the bactericidal action of the serum Heat-inactivated serum (Δs) and seminal plasma alone or in combination were not bactericidal.

FIGURE 4   Opsonization-phagocytosis killing assay for N. gonorrhoeae strain 867 with serum deficient in C8 as the antibody and complement (opsonin) source. The serum (s) with polymorphonuclear cells (PMN) yielded opsonization with phagocytosis and killing. Addition of seminal plasma (♂) diluted 1:5 inhibited the s plus PMN killing. The serum and heat-inactivated (56°C, 30 min) serum had no killing activity. PMN and ♂ alone or together (not shown) also had no killing effect.
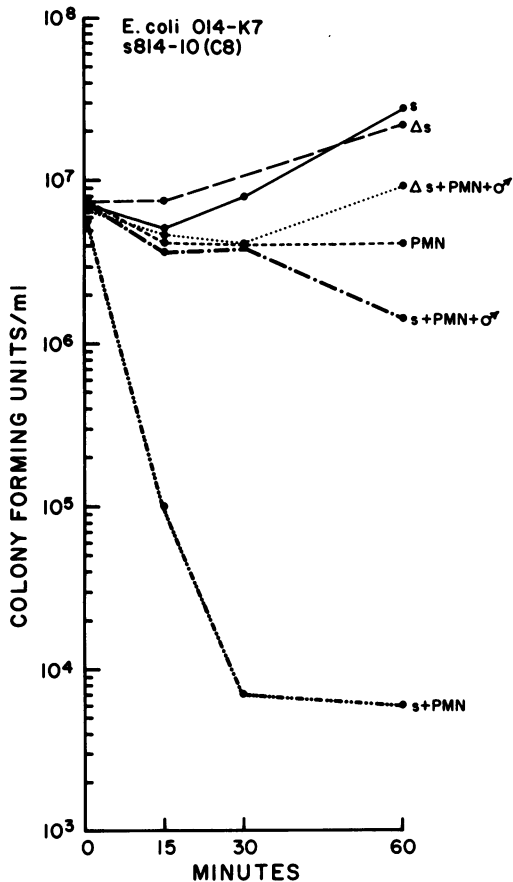
FIGURE 5 Opsonization-phagocytosis killing assay for *E. coli* 014-K7 using serum deficient in C8 as the antibody and complement (opsonin) source. The serum (s) plus polymorphonuclear cells (PMN) yielded opsonization with phagocytosis and killing. Seminal plasma (♂) diluted 1:5 inhibited the s plus PMN killing. Serum, heat-inactivated (56°C, 30 min) serum (Δs) and PMN alone or in combinations had no killing activity. Seminal plasma alone (not shown) also had no killing activity.

trols. In the stains of the mixtures with C8-deficient serum as the active opsonin, the bacteria appeared to have been ingested by the polymorphonuclear cells. In the mixtures with serum and seminal plasma and in the controls, the bacteria were often at the periphery of the polymorphonuclear cells and did not appear to have been ingested.

Seminal plasma effect on polymorphonuclear cell chemiluminescence. The preopsonized *E. coli* yielded a peak chemiluminescence response of 10^6 cpm after 90 min incubation. Addition of 10% seminal plasma reduced the peak response by 68%, to 3.2×10^5 cpm. When nonpreopsonized *E. coli* were used and fresh serum was added during the reaction a lower peak of chemiluminescence response was obtained, but a similar percentage reduction occurred with addition

of seminal plasma; the response went from 4.1×10^5 to 1.3×10^5 cpm.

The phorbal myristic acid induced chemiluminescence also was inhibited by seminal plasma. The peak response of 5×10^6 cpm was reduced 68% to 1.6×10^6 cpm by 1% seminal plasma; there was 90% reduction to 4.1×10^5 cpm by 10% seminal plasma.

Seminal plasma effect on antibody attachment and function. Seminal plasma did not block the attachment of IgG, IgM, and IgA to gonococci or *E. coli*. The fluorescent antibody procedure showed no seminal plasma effect on antibody attachment when serum was diluted up to 1:1,024. The radioiodinated antibody procedure showed no effect with the serum diluted up to 1:10,000. Differences in end points were noted for the different immunoglobulins, but for each immunoglobulin class, the untreated, and seminal plasma treated specimens were the same.

IgM plus Cd was bactericidal at a concentration of 1.5 µg/ml. The IgG was bactericidal at a concentration of 33 µg/ml. The bactericidal action of complement with either IgM or IgG was inhibited by 1:20 dilutions of seminal plasma. Cd with IgA was not bactericidal.

The gonococci and *E. coli* pretreated with C8-deficient serum followed by seminal plasma treatment showed 3+ fluorescence when stained with anti-C3 fluorescein-labeled antibody. Similarly, pretreatment of the bacteria with seminal plasma followed by C8-deficient serum treatment also yielded 3+ fluorescence when treated with the anti-C3 antibody.

Seminal plasma effect on CH_{50}. The CH_{50} activity of normal human serum was reduced ~25% by addition of seminal plasma diluted 1:5. Preincubation of the sheep erythrocytes and seminal plasma with washing of the erythrocytes did not yield a change in CH_{50} activity. The sequence of addition or preincubation of seminal plasma with the other components of the hemolytic complement assays did not affect the decrease in CH_{50}. Seminal plasma appeared to have a significant inhibitory effect on complement activation. The detailed analysis of seminal plasma-mediated complement inhibition is presented separately (21).

Characteristics of the seminal plasma bactericidal inhibitor. Chromatography of crude seminal plasma on Sephadex G-25 yielded two major protein peaks. The bactericidal inhibitor substance was present in pooled fractions from both peaks and in dialysates of both pooled fractions. The bactericidal inhibitor substance also was dialyzable from whole seminal plasma.

Whole seminal plasma diluted to protein concentrations of 0.5–0.004 mg/ml inhibited the bactericidal action of complement plus immune serum depending upon the concentration of immune serum used.

The bactericidal inhibitory activity of seminal plasma was not modified by the storage at 25°, 4°, or −13°C as tested by daily analysis for a period of 3 d. It was stable

TABLE III
Phagocytic Indices of N. gonorrhoeae, E. coli, and S. aureus Opsonized
with C8-deficient Serum Plus Polymorphonuclear Cells without
or with Added Seminal Plasma

	N. gonorrhoeae (7)	E. coli (3)	S. aureus (5)
	%		
S(C8) + PMN	48.4±7.6* ⎤	53.0±8.2* ⎤	51.8±8.8‡ ⎤
S(C8) + PMN + ♂	26.6±15.1* ⎦	19.7±3.8* ⎦	43.0±12.3‡ ⎦
ΔS(C8) + PMN + ♂	22.6±10.3	22.3±12.7	23.6±11.2
PMN + ♂	28.1±13.0	17.0±4.2	10.2±6.5

S(C8), C8-deficient serum; PMN, polymorphonuclear cells; ΔS heated serum. Phagocytic indices were determined by counting Wright-Giemsa-strained PMN with associated bacteria. Numbers of experiments are in parentheses. The phagocytic index was defined as the percentage of PMN with associated bacteria. Results are expressed as mean±SD for each organism. Seminal plasma blocked the association of the gram-negative bacteria with PMN, but no significant reduction in S. aureus-PMN association was seen. In separate experiments, ΔS plus PMN or PMN alone also had very few associated bacteria.
* $P < 0.01$ and >0.005.
‡ $P < 0.4$ and >0.2.

for at least five freeze ($-13°C$)-thaw cycles. The inhibitory activity was stable at 56°C for 30 min, but was destroyed by 100°C for 10 min.

The trypsin, soybean trypsin inhibitor, chymotrypsin, and protease each affected the viability of N. gonorrhoeae but not E. coli. However, none affected the seminal plasma bactericidal inhibitory activity.

Addition of 1.0 mM dithiothreitol yielded no change in the seminal plasma bactericidal inhibitor activity. Purified spermine (7.8–500 μg/ml) or spermidine (0.78–50 μg/ml) alone or in combination added to the N. gonorrhoeae serum bactericidal system had no inhibitory effect on bacterial killing.

DISCUSSION

Human seminal plasma contains a potent inhibitor of serum or antibody complement-mediated bactericidal action on N. gonorrhoeae and N. meningitidis. The bactericidal action of serum on gram-negative rods also was inhibited.

Seminal plasma inhibited the in vitro opsonization and polymorphonuclear cell killing of N. gonorrhoeae and E. coli, but not S. aureus. Analysis of the phagocytic index data suggests that a large portion of the seminal plasma inhibition occurred during the steps of opsonization. In the presence of seminal plasma, very few N. gonorrhoeae or E. coli were associated with polymorphonuclear cells. However, we cannot exclude the possibility that factors in seminal plasma were internalized by the polymorphonuclear cells resulting in selective effects on the intracellular killing of the gram-negative bacteria. That seminal plasma did not non-

specifically inhibit the ability of polymorphonuclear cells to kill bacteria is indicated by the lack of effect on killing of S. aureus. The observation that seminal plasma did not inhibit the opsonization and intracellular killing of S. aureus is interesting, but unexplained.

Seminal plasma also inhibited the polymorphonuclear chemiluminescence response to opsonized bacteria and phorbol myristic acid. We feel this observation does not explain the inhibition of opsonization/polymorphonuclear cell killing. For example, seminal plasma contains ascorbate in sufficient concentrations to modify polymorphonuclear cell metabolism (22, 23). However, ascorbate does not prevent polymorphonuclear cell killing of bacteria (24) and may enhance the killing in Chediak-Higashi syndrome (25). Seminal plasma is a biochemically complex material and it is likely that its effects on polymorphonuclear cells are not simple.

Seminal plasma did not inhibit the attachment of immunoglobulins to gonococci and E. coli, but did inhibit bactericidal action when hypogammaglobulinemic complement donor serum was used with immune serum or purified IgM or IgG.

Seminal plasma significantly reduced serum whole hemolytic complement activity. Hemolytic assays are far more sensitive than bactericidal or opsonization assays as measures of complement function. Addition of 100 U of C8 to C8-deficient serum repletes CH_{50} activity, but 10,000 U are required to replete bactericidal activity (7). It is likely the seminal plasma-induced reduction in CH_{50} activity is sufficient to explain the inhibition of bactericidal and opsonic activities. This possibility is strongly supported by the

observations that hemolytic titers of C3 in *N. gonorrhoeae* and *E. coli* bactericidal reactions are markedly reduced in the presence of seminal plasma, and no bactericidal action occurs (21). Seminal plasma also inhibits alternate pathway activity, cleaves factor B, depresses the functional capacity of C1 and C3 by more than 50% and reduces the functional activities of some erythrocyte antibody complement-stable intermediates. Singly or collectively, these seminal plasma effects on complement function could inhibit antibody complement-mediated bactericidal and opsonic activities. The detection of bound C3 by the fluorescein-labeled anti-C3 antibody is interesting, but does not negate the probable anticomplementary effect of seminal plasma. The C3 detected on the surface of the bacteria could have been an opsonically inactive C3 fragment such as C3d. This would not have been differentiated by the fluorescein-labeled antibody.

The chromatography and dialysis characteristics of the seminal plasma inhibitory factor indicate that it is a small molecule, probably <20,000 mol wt. However, the chemical nature of the bactericidal inhibitor remains unknown. It is not a polyamine, based on the experiments using purified polyamines and does not appear to have sulfur containing residues affected by dithiothreitol. It also is unlikely the inhibitory effect is due to chelation. The calcium and magnesium concentrations in the buffered medium 199 should have been far in excess of the amount that could be removed by seminal plasma chelation effects.

The susceptibility of the seminal plasma inhibitory factor to heating at 100°C is compatible with the inhibitory substance being a protein. Seminal plasma contains a large number of proteins including enzymes and enzyme inhibitors—many of which are low molecular weight (22, 26–29). That we were not able to modify the seminal plasma bactericidal inhibitory activity using a limited number of proteases and a protease inhibitor does not exclude the possibility that the bactericidal inhibitor is a low-molecular weight protein with enzyme or enzyme inhibitory action. Also, it is possible that there is more than one seminal plasma inhibitory substance.

The seminal plasma bactericidal inhibitor appears to be different from the inhibitor of lymphocyte activation. The latter has a molecular weight of >100,000 (3), whereas the bactericidal inhibitor activity can be found in both the low- and high-molecular weight fractions from Sephadex G-25 columns. The presence of the bactericidal inhibitor in both fractions suggests that it may be bound to a higher molecular weight substance or that it may be the cleavage product of a higher molecular weight substance.

C3 has been detected in cervical mucus (30, 31) and it has been suggested that C3 is synthesized in vitro by vaginal mucosa (32). CH_{50} activity equivalent to 11.5% of serum levels has been found in human mid-cycle cervical mucus (33). Both serum and secreted immunoglobulins are present in the female genital tract (27, 30, 31, 34). The combination of complement and immunoglobulins in the cervix could function in a protective manner (35, 36) and may result in the generation of chemotaxins and in opsonization of *N. gonorrhoeae*. These potentially protective actions of complement and antibodies in the female genital tract would be effectively blocked by seminal plasma.

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