Interactions of *Neisseria gonorrhoeae* with Human Neutrophils

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The hallmark of acute gonococcal urethritis in males is the presence of gram-negative intracellular diplococci. Not only has this observation served as a diagnostic indication of gonorrhea, it has also suggested that *Neisseria gonorrhoeae* has the capacity to survive the massive inflammatory response of the host during infection. Thus, the interaction of gonococci with human phagocytic cells has both clinical and experimental significance.

It is the purpose of this review to discuss experiments performed during the past several years that deal with the interactions between gonococci and phagocytes. For the most part, this review will concentrate on polymorphonuclear leukocytes (PMNs), since their presence in purulent exudates is most remarkable and has been studied vis-à-vis *N. gonorrhoeae* to a greater extent than that of mononuclear phagocytes. Moreover, since gonococci are obligate human pathogens and since no cost-effective animal model mimics human infections, only experiments with human materials will be reviewed, except when comparisons help us to better understand the molecular events involved in gonococcus-PMN interactions.

Intraleukocytic killing of gonococci is the final event in the process of interest. However, killing requires certain sequential events which, at each step, can be resisted by gonococci. For instance, gonococci may resist phagocytes by killing them, inhibiting their chemotaxis, resisting ingestion, or inhibiting or surviving intraleukocytic killing mechanisms. Accordingly, this review is divided into three sections: extracellular events, cell surface events, and intracellular events. Although gonococci and other pathogenic bacteria are subjected to similar processes, research dealing with gonococci and PMNs has provided new information regarding phagocytosis, phagocytic killing, and the biology of gonococci. Therefore, we have sought to identify how studies dealing with gonococci and PMNs have helped to advance not only the field of gonococcal pathogenesis but also the biology of PMNs. Where there appear to be answers to certain questions, we have provided them. Where questions remain, we have pointed them out in a manner that we hope will spur new approaches to an old problem. Finally, as is often the case with gonococci, results and conclusions from many early experiments that examined the interaction of gonococci and PMNs must be reevaluated as the genetics, physiology, and structure of *N. gonorrhoeae* have become better understood.

EXTRACELLULAR EVENTS

In considering the extracellular events that take place during the initial phases of phagocytosis, three topics are important: chemotaxis, opsonization, and cytotoxicity. The first two favor the host, whereas the last (bacterium-mediated cytotoxicity) may help gonococci evade the initial

defensive response of the host. Only a limited number of studies provide an indication of the events occurring prior to the physical interaction of gonococci with the surface of the phagocyte.

Chemotaxis

Chemotaxis is defined as the migration of phagocytic cells up a concentration gradient that often originates from infected areas. It was one of the first biological processes noted in immunological research and was central to the Metchnikoff (66) phagocytic theory of host defense. The capacity of PMNs to infiltrate tissues and fluids in response to chemotactic signals may shed light on the massive presence of PMNs in acute gonococcal urethritis. Conversely, down-regulation of the chemotactic process may help to explain asymptomatic infections. Thus, it is important to know the nature of the chemotactic signals recognized by PMNs during gonococcal infection and whether they differ during the course of acute, asymptomatic, or invasive forms of disease.

The principal chemotactic signal relevant to the migration of PMNs to gonococci is C5a, which is generated by cleavage of C5 by C5 convertase. This complement-derived neutrophil chemoattractant has been shown by Densen et al. (24) to be markedly increased in serum exposed to serum-sensitive as opposed to serum-resistant gonococci. This is important since serum-sensitive gonococci cause significantly greater inflammatory symptomatology in women with acute salpingitis than the low-level symptomatology seen in individuals with disseminated gonococcal infections that are typically caused by serum-resistant strains (87). Thus, the acute nature of salpingitis may be directly related to the capacity of serum-sensitive strains to increase the magnitude of C5a generation. This would stimulate PMN migration, leading to the severe inflammation seen in this form of gonorrhea.

Intact and sonicated gonococcal peptidoglycans (PG) activate the classical complement pathway in an antibody-dependent manner (79), thereby generating C5a. However, lysozyme digestion of sonicated PG abrogates this activity, but PG that is O acetylated is resistant to such digestion (90). Accordingly, PG fragments containing glycosidic linkages that are liberated in vivo and are recognized by antibody to PG should have a bearing on inflammation.

Cytotoxicity and Interference with PMN Functions

Microorganisms, through the production of leucocidins, have the ability to resist phagocytes by killing them prior to being ingested. Microscopic evidence suggests that some PMNs may fall victim to toxic substances released by gonococci. Visualization of purulent exudates reveals not only abundant numbers of intact PMNs but also phagocytes that appear to be damaged or disintegrating (18, 19, 68, 72). However, since the fully mature PMN has a half-life of about

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8 h, it is difficult to distinguish whether such PMNs have been attacked by gonococcal cytotoxic agents or are merely succumbing to old age. Certain gonococcal cell envelope components may participate directly in damaging PMNs. Gonococci, like other gram-negative bacteria, release outer membrane vesicles that may indirectly serve to deliver toxic compounds. Hence, potential cytotoxic agents released by gonococci might be individual factors released into the environment or outer membrane (OM) components released as part of membrane vesicles. Casey et al. (19) presented evidence that gonococci produce a cytotoxic substance with the ability to lyse PMNs. The substance did not appear to be lipopolysaccharide (LPS), which is known to damage fallopian tube tissue (21), or protein. No further reports regarding the nature or mechanism of action of this cytotoxin have appeared. Its presence should be confirmed, and, if it is found, its distribution among gonococcal isolates should be evaluated. Its mode of action should also be determined.

Studies that have investigated the structure and function of the outer membrane on protein (OMP) protein I (PI) (5, 6, 43, 45), however, provide a better example of how a gonococcal protein might modulate PMN function. PI is known to directionally insert into eucaryotic membranes, and this action may have dire consequences for PMNs (43, 64, 135). For instance, PI depolarizes the PMN membrane, resulting in inhibition of degranulation; this suggests that it could influence PMN structure and function. Since intracellular killing of gonococci requires the development of a phagolysosome (25), the inhibition of degranulation would prohibit the release of toxic compounds into the phagocytic vacuole. This may help to explain why some intraphagosomal gonococci resist the antimicrobial action of lysosomal components (see below).

Opsonization

What role do opsonins (12) play in phagocytosis of gonococci in vitro, and are opsonins produced and active in vivo? Gonococcal variants possessing certain PII proteins are readily phagocytized and killed in vitro (33, 53, 54, 83, 84, 129). With these strains, opsonins present in serum may have little effect on the outcome of gonococcus-PMN interactions. On the other hand, gonococci lacking PIIs or possessing nonadherent PIIs or, perhaps, strains displaying certain pilus chemotypes are not recognized by PMNs in the absence of opsonins.

Fresh human serum contains opsonic anti-PI immunoglobulin G (94, 95). Fresh convalescent-phase human serum samples collected during localized, disseminated, and uncomplicated gonococcal infections are opsonic and contain immunoglobulin G (11, 62) to most surface-exposed components, including PI (34, 63), PII (97), PIII (63, 86), the iron-regulated proteins (34), pili (34, 102), H.8 (4), and as yet uncharacterized components (34, 136). Such antibodies are also present in cervical and urethral secretions to various degrees (47, 65, 124).

In human PMN chemotaxis studies, Densen et al. (24) showed that complement deposition on disseminated isolates was less than and slower than that observed on local isolates, when incubated in 10% fresh human serum. Heat- or ethylenediaminetetraacetic acid (EDTA)-treated serum inhibited complement deposition on all strains and abrogated the subsequent release of the chemoattractant, C5a. Ross and Densen (92) further showed that serum-sensitive strains are more rapidly and completely ingested and killed by human PMNs than are serum-resistant strains in nonbacte-

ricidal C8-deficient serum or C8-depleted normal serum. More specifically, phagocytic killing of serum-resistant strains was unaffected by serum; in the presence of PMNs, it retained about 65% of its viability in the presence of 0.01 to 10% serum. In contrast, phagocytic killing of serum-sensitive strains was strictly dependent on the serum. An alternative explanation of the data, however, is that the representative serum-sensitive strain was consistently PII⁻, whereas the serum-resistant strain was PII⁺, thus confounding the interpretation of the results. Others find no differences in the phagocytic killing of unopsonized, nonpiliated PII⁺ strains of serum-sensitive and serum-resistant strains (S. H. Fischer and R. F. Rest, unpublished data).

Opsonophagocytosis therefore appears likely in vivo, although we are not aware of studies investigating such processes. It is unknown which of the apparent opsonins is the most relevant for protection of the host.

SURFACE EVENTS

Gonococcal Surface Components Implicated in Gonococcus-PMN Interactions

Antiphagocytic activities and components. (i) PI. In a series of elegant studies, Blake and co-workers showed that PI translocated from the gonococcal OM to erythrocyte and artificial membranes (135) and associated with PMNs (64). PI forms ion channels in these membranes and selectively inhibits exocytosis from human neutrophils without inhibiting superoxide generation (43). Whether PI affects the ability of gonococci to survive neutrophil killing is not known.

(ii) Pili. Although pili are unquestionably important in initial interactions with host epithelial cells and in initiating gonococcal infection (46, 51, 52, 58, 111, 116, 118), their role in gonococcal interactions with PMNs remains open to question, and older data and conclusions must be reevaluated. With few exceptions, earlier studies showed that piliated gonococci (from type 1 or type 2 colonies, as originally described by Kellogg et al. [51, 52]) resisted neutrophil phagocytosis, whereas nonpiliated organisms, from colony type 3 or type 4, were ingested (27, 41, 58, 71, 80, 122, 123). However, more recent studies, and some earlier ones, have used gonococci with a better-defined PII content, as determined by a combination of colony morphology (59, 115), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of outer membrane preparations (33, 54, 83, 112–116, 128, 129). The results of these studies have suggested that in the presence of PII, pili have little effect on the degree to which gonococci adhere to, stimulate, or are killed by human PMNs. Thus, PII appears more important than pili in mediating gonococcal interactions with PMNs, including adherence and modulation of phagocytosis (59, 60).

The story, however, is not complete. Pili undergo structural, antigenic, and functional alterations owing to antigenic variation (40, 103). There are no reports about the interaction of gonococcal pilus variants with PMNs. It is known that different pilus types confer upon gonococci different adherence properties for epithelial cells in culture (61). Recent information suggests that the interaction of gonococci may not be mediated by the major pilus subunit, pilin, but rather by as yet poorly defined pilus-associated proteins (67, 109) or proteins coexpressed in the complex transition from colony type 1 to colony type 4 (55). The molecular mechanisms by which pili mediate gonococcal interactions with human PMNs is far from completely understood, but it

is known (117) that piliation does not influence phagocytic killing.

(iii) **Less well-defined events and components.** In vitro growth of nonpiliated opaque (PII⁺) gonococci in the presence of heat-inactivated normal human serum decreased their association with PMNs (9), reinforcing the observed need for opsonins to obtain efficient phagocytic killing. Gonococci isolated directly from human material or from guinea pig subcutaneous chamber fluid resisted phagocytic killing to a greater extent than did those grown in vitro (18, 126, 127, 134). In vivo resistance factors might be lost or altered upon subculture, as is the case for the antigenic and chemical structure of LPS (23, 96). Rosenthal et al. (91) identified an EDTA-sensitive antiphagocytic surface component of gonococci that has not been further studied. In addition to resisting phagocytosis or intracellular killing, gonococci might elaborate components toxic to neutrophils (19, 21, 43). Thus, from metabolic processes to surface structures and released toxic materials, gonococci possess a wide array of antineutrophil systems.

Adhesins and phagocytosis-promoting activities and components. (i) **PII.** There are at least three reasons why many early studies investigating the role of pili and other gonococcal surface components in the interaction with PMNs should be reevaluated. First, contrary to original observations and misconceptions, the possession of a particular PII does not necessarily confer upon gonococci the ability to form opaque colonies. Thus, transparent colonies can contain PII⁺ gonococci, or, conversely, PII⁺ gonococci can form transparent colonies (59, 115). Second, not all PII proteins confer upon gonococci the ability to adhere to, stimulate, and be phagocytically killed by PMNs (33, 60). Thus, colony opacity and adherence are unrelated, except under very well defined conditions. The leukocyte association protein (LAP) described by Swanson et al. was most probably a "transparent" PII, which is why its initial identification was so elusive (see below). Indeed, leukocyte association protein shares peptides with certain PIIs (114). Third, considering the high rate of pilin and PII antigenic variation (3, 40, 103) and the practical experience of many investigators, it is doubtful that the piliated and nonpiliated "isogenic" variants used in earlier studies possessed the same PII (or pilin subunits) throughout the period of study.

PMNs and gonococci are found in close association in vivo (31, 68, 72, 130, 131). They also adhere to each other in vitro in the absence of antibody. The leukocyte association protein, subsequently identified as a PII (114), was the first surface component other than pili to be studied in the adherence of gonococci to human PMNs. In the absence of opsonins, piliated or nonpiliated gonococci lacking PII adhered poorly to PMNs, were not engulfed or killed by PMNs, and induced only a low-level PMN oxidative response, compared with those possessing PII (33, 84, 129). Interestingly, PII also appeared to confer upon gonococci the ability to be phagocytically killed by *Trichomonas vaginalis* (32).

Which part of the PII molecule is involved in binding to PMNs? The various PII molecules synthesized by a particular strain generally have identical amino acid sequences except for a short semivariable (SV) region near the amino terminal and two longer hypervariable regions (HV1, [about 20 amino acids], and HV2, [about 35 amino acids]) in the carboxyl half of the PII molecule (20, 107). There are several variants of each HV region, e.g., HV1-1, HV1-2, HV1-n, and HV2-1, HV2-2, HV2-n. Differences in the SV region are limited to small changes, whereas the HV1 and HV2 regions

show essentially no homology between or within themselves.

Several, but not all, monoclonal antibodies (whole or Fab fragments) directed against epitopes in HV2 block the ability of PII⁺ gonococci to adhere to and stimulate PMNs (84, 129; C. Elkins and R. F. Rest, submitted for publication). The HV1 region does not seem to be directly involved in binding, since variants FA 1090 PIIa, which does not bind to PMNs, and FA 1090 PIIb, which does bind to PMNs, vary only in their HV2 region (20, 33; Elkins and Rest, submitted). Besides possessing different HV2 regions, PIIb (the adherent PII) possesses seven amino acids immediately adjacent to the HV2 region that PIIa (the nonadherent PII) lacks (20). Thus, an area near HV2 may be involved in gonococcal adherence to PMNs.

It has been suggested that PII mediates lectinlike interactions of gonococci with PMNs (84) and that opacity-associated PIIs bind gonococcal LPS (7). However, carbohydrate-specific binding of purified PII to HeLa cell membrane proteins was not observed (2). Such experiments have not been reported with proteins from PMN membranes. In addition, some of the observations by Rest et al. (84) concerning the ability of mannose to inhibit gonococcus-induced PMN chemiluminescence may have been artifactual, since further studies revealed that mannose inhibits PMN oxidative metabolism in response to several stimulating agents (82). PIIs have been strongly implicated in adherence to epithelial cells (1, 48, 110). Whether different PIIs modulate adherence to different cell types remains to be determined.

(ii) **LPS.** In adherence studies with PMNs and piliated gonococci (whose PII content was not defined), Kinane et al. (53) showed that additional lectinlike interactions might occur, with the lectin on the neutrophil and the carbohydrate on the gonococcus. This suggests a role for LPS, or an unidentified gonococcal glycolipid or glycoprotein, in the adherence of gonococci to PMNs. With the recent description of frequent antigenic, structural, and functional changes (similar in frequency to pilin and PII antigenic variation) in the gonococcal LPS, such observations take on a new significance (26, 96).

Periodate oxidation abrogates gonococcal adherence to PMNs (54; Elkins and Rest, submitted). Since this is true for nonpiliated, PII⁺ organisms, it can be concluded that PII, which does not contain carbohydrate, contains a periodate-sensitive portion, perhaps containing the periodate-sensitive amino acid serine, threonine, or tyrosine (39, 132). Alteration of LPS by periodate does not appear to be responsible for these observations, since a gonococcal LPS mutant, WS 1 (100), which contains few or no hexoses, also binds to PMNs in a strictly PII-dependent manner (Elkins and Rest, submitted). Further studies have shown that binding of nonpiliated PII⁺ gonococci to PMNs (i) is temperature dependent, with no binding occurring at 0 to 5°C; (ii) is independent of gonococcal viability, since gonococci killed by gentamicin or ultraviolet light bind as well as viable gonococci (Elkins and Rest, submitted); and (iii) is inhibited by treatment of gonococci with trypsin, chymotrypsin, proteinase K, or V8 protease.

Characterization of Gonococcus-PMN Interactions and Possible Receptors

Few studies have characterized the molecular mechanisms involved in gonococcus-PMN interactions or investigated the structure of the PMN receptor(s) for gonococcal

adhesins. Adherence of gonococci to PMNs is not inhibited by protease treatment of PMNs (Elkins and Rest, submitted). On the contrary, PMNs treated with proteinase K show a 5- to 10-fold increase in the binding of gonococci (C. Elkins, C. Farrell, and R. F. Rest, unpublished data). Several PMN secretagogues, including phorbol myristate acetate, the calcium ionophore A23187, and the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (in the presence of 5 g of cytochalasin per ml), all increase, in a dose-responsive manner, the ability of PMNs to bind non-piliated PII⁺, but not PII⁻, gonococci. Furthermore, cells of the promyelocytic cell line HL-60 do not bind such gonococci, regardless of their state of differentiation or the presence of secretagogues (Elkins et al., unpublished).

Recently, Stromberg et al. (109) showed that gonococci, regardless of piliation or PII content, adhere to several glycolipids, including lactosyl-, isoglobotriaosyl-, gangliotriaosyl-, and gangliotetraosylceramides. Whether these glycolipids play a role in binding of gonococci to PMNs or to epithelial cells is not known, although the last two, also known as asialo-GM₂ and asialo-GM₁, respectively, have not been identified in neutrophils (36, 37, 121). Such results gain importance in light of new studies defining gonococcal pilus-associated (nonpilin) proteins (68). Perrollet and Guinet (78) described a 65-kilodalton gonococcal outer membrane protein with maltose- and D-glucosamine-binding activities. Its identity with OMP-macromolecular complex (44) and its role in adherence to host cells is unknown. Wiseman et al. (133) have suggested that piliated (colony type 1) gonococci agglutinate human erythrocytes (13, 57, 80) as a result of interactions with D-galactose-containing erythrocyte surface-exposed glycoconjugates. In studies with lectin-resistant Chinese hamster ovary cells (29), Gubish et al. (42) observed that isolated pili adhere to parental cells much better than to the mutant cells lacking sialic acid, galactose, and N-acetylglucosamine in their cell surface glycoconjugates. Results obtained by Pearce and Buchanan support the idea that gonococcal pili adhere to glycosphingolipids of host cells (77). Such interactions are not inhibited by mannose or other monosaccharides. A common thread to all these studies is the dependence of gonococcal binding to host cells on galactose-containing glycoconjugates, probably glycolipids.

INTRACELLULAR EVENTS

Gonococcal Survival within PMNs

Whether gonococci survive within PMNs has been the subject of controversy, with no clear-cut answer yet available. Perhaps no other issue in this field has generated more conflicting data. The intracellular fate of gonococci is clearly an important issue, given that protective antibodies resulting from vaccination will probably have to be opsonic.

Densen and Mandell (25) established that phagolysosomal formation was required for phagocytic killing of gonococci. No evidence to date has been published that intracellular gonococci inhibit phagosome-lysosome fusion or that certain gonococci escape from the phagolysosome. Accordingly, if gonococci are to survive within PMNs, they apparently must resist antimicrobial systems that operate in the phagolysosome.

What is the conflicting evidence regarding the intracellular fate of gonococci, and how can the controversy be explained? First, it is important to note that differences in phagocytic killing assays (monolayer versus suspensions of PMNs), strain heterogeneity (isolates from localized versus

disseminated gonococcal infection), the efficiency with which attached gonococci are eliminated by antimicrobial agents, and gonococcal growth conditions (log-phase versus stationary-phase gonococci, broth grown versus agar grown, aerobic versus anaerobic conditions) are likely to influence the rate and extent of killing. Hence, assessment and comparison of survival data from different laboratories are difficult. Second, it is very important to remember that phagocytosis assays performed in the laboratory may only slightly resemble the events occurring in vivo and the environmental conditions in tissues or blood. Although this is always a problem in research on microbial pathogenesis, it is especially true for gonococci, given the highly variable surface structures and response to environmental stresses.

The greatest proponents of the intracellular survival of gonococci are Smith and co-workers in England. In a series of papers (17-19, 73-76, 126, 127, 134) starting in the mid 1970s and continuing to the present, this group presented data suggesting that a percentage of gonococci not only survive but also replicate within PMNs; replication was inferred on the basis of the capacity of penicillin to kill ingested gonococci (126), with the rationale that penicillin kills only growing bacteria. Moreover, such intracellular survival of gonococci is enhanced if gonococci are first cultivated in vivo by using the guinea pig chamber model (127). Such gonococci are postulated to more closely resemble, in an antigenic and physiological sense, gonococci growing in humans. In support of this, Parsons et al. (73-75) recently reported that a surface-exposed 20-kilodalton OMP promotes intracellular growth and survival of gonococci. This OMP contains fatty acids (ca. 5.7% of the total protein content) and is rich in glutamic acid. Evidence that this lipoprotein confers on gonococci increased resistance to intracellular killing is based, in part, on the observation that a monospecific antiserum against it reduces gonococcal resistance to intracellular killing. Clearly, this antigen is of interest and should be studied in more detail at both the biochemical and molecular levels. How it confers gonococcal resistance to killing systems in phagolysosomes will be of interest. Of additional interest is how in vivo growth stimulates its synthesis.

Not all researchers have observed intracellular survival and growth of gonococci (25, 27, 41, 58, 83, 95, 122). These investigators observed that when an inoculum of gonococci was allowed to interact with PMNs in either monolayers or suspension, the ingested gonococci were rapidly killed. Any gonococci that survived, however, were suspected to be attached to PMNs in a manner that enabled them to resist the extracellular antimicrobial agents typically added to eliminate them. Accordingly, the question of intracellular survival of gonococci remains controversial. However, in our opinion, the major difference between the studies by Smith and co-workers and those of others is that the former group has often used gonococci directly from clinical material, while the latter has not.

The observations of Smith and co-workers may ultimately prove to be correct in that survival of gonococci in vivo is greater than that observed in *in vitro* phagocytosis assays. Recent support for the concept that some intracellular gonococci survive comes from the work of Casey et al. (16). In a study to test whether anaerobically maintained PMNs killed gonococci as effectively as aerobically maintained PMNs did, they used pyocin 103 to kill piliated gonococci attached to PMNs. This approach was needed to rigorously distinguish attached from ingested gonococci (120). Pyocin 103 exhibits potent gonococcidal action and is not phagocytized,

thus proving to be an effective extracellular killing agent. They observed that approximately 2% of the total ^{14}C -labeled, piliated gonococci ingested by PMNs survived for at least 165 min. Two important conclusions can be drawn from these experiments. First, in the absence of oxygen, PMNs kill 98% of the ingested gonococci, thereby implicating oxygen-independent antimicrobial mechanisms as contributing significantly to intraleukocytic killing of gonococci. Second, despite such effective intraleukocytic killing of gonococci, a small but reproducible number survive.

Oxygen-Independent Killing of Gonococci by PMNs

The finding (16) that an oxygen-independent antimicrobial mechanism(s) contributed significantly to intraleukocytic killing of gonococci was consistent with the earlier work of Rest et al. (83), who showed that PMNs obtained from normal healthy donors and patients with chronic granulomatous disease displayed identical phagocytic killing capacities. This observation was significant because chronic granulomatous disease renders phagocytes incapable of generating toxic O_2 radicals necessary for oxidative killing systems (reviewed in part in reference 104). Hence, intraleukocytic killing of gonococci by chronic granulomatous disease PMNs is due entirely to a nonoxidative process.

It is also important to note that neutrophils derive all their energy through glycolysis (104). Hence, an anaerobic environment places no constraints on their energy needs. In fact, it is tempting to speculate that the lack of toxic oxygen radicals within a PMN may prolong its life, or at least make it more enjoyable.

The illuminating work of Britigan et al. (9–11) suggests that even under aerobic conditions, gonococci may be killed by normal PMNs by nonoxidative processes. They showed that gonococci exposed to serum- or phagocyte-derived lactate rapidly consumed the available molecular oxygen. Thus, gonococcal oxygen consumption would deplete the oxygen available to PMNs in a microenvironment, thereby reducing their capacity to mount an oxidative burst.

The emerging realization that gonococci survive and grow under anaerobic or hypoxic conditions (15, 49, 50, 56) and coexist with strict anaerobes in vivo (35) is another reason why the nonoxidative antimicrobial system of PMNs is an important topic for study. Largely through the work of Clark and co-workers (55, 56), we know that anaerobic conditions can be tolerated if gonococci are provided with a surrogate electron acceptor such as sodium nitrite and that anaerobiosis regulates the synthesis of gonococcal OMPs. Hence, gonococci growing anaerobically in vivo may little resemble the same strain propagated in vitro in broth or on agar in an oxygenated environment. How might anaerobic conditions influence gonococcal pathogenesis, given that the repertoire of surface antigens might change? First, in the absence of a surrogate electron acceptor, gonococci are in a state of bacteriostasis. Casey et al. (15) found that this resulted in gonococcal resistance in vitro to antimicrobial granule proteins. Addition of sodium nitrite, however, permitted growth in anaerobic broth and resulted in a level of granule protein susceptibility similar to that in aerobically grown gonococci. This is consistent with the earlier work of Rest and co-workers (14, 81, 88, 89), who showed that granule proteins kill actively growing gonococci better than they kill stationary-phase gonococci.

The antimicrobial action of certain cationic granule proteins is thought to be a major determinant of nonoxidative killing of bacteria within phagolysosomes (104). These toxic

proteins are stored in the cytoplasmic granules and are released into the vacuole, where they rapidly bind to the microbial surface. To date, several proteins have been isolated from extracts of granules and shown to possess antimicrobial action in vitro (38, 69, 98–101, 104). Those possessing antigonococcal activity are CAP 37, CAP 57, and cathepsin G (98–101). The low-molecular-weight defensins (38), having potent antibioticlike activity against several bacteria, fungi, and viruses, have surprisingly little (if any) antigonococcal activity in vitro.

Shafer et al. (98–101) have studied extensively the antigonococcal activity of cathepsin G and concluded that it is a likely participant in the killing of gonococci in phagolysosomes. Although this protein is a serine protease with chymotrypsinlike specificity (69, 93), it kills gonococci and other pathogens by a mechanism independent of proteolysis; neither boiling for 5 min nor treatment with the potent serine protease inhibitor diisopropyl fluorophosphate diminishes the bactericidal action of cathepsin G (69, 70, 100, 101). Thus, one can exclude proteolytic damage to the outer membrane as a mechanism of killing. The highly cationic nature of cathepsin G ($\text{pI} > 12.5$) is a probable reason why it binds avidly and in a saturable manner to gonococci (98). A single diplococcus has approximately 10^5 binding sites for cathepsin G. These binding sites are masked by LPS, since a mutation which truncates LPS enhances gonococcal susceptibility to cathepsin G and increases the specific binding of cathepsin G 10-fold. Analysis of the saturable binding of cathepsin G to gonococci revealed a binding constant of 10^8 M^{-1} .

PI and PIII appear to bind cathepsin G (98, 99). This was first appreciated when the degradation of PI and PIII by enzymatically active cathepsin G was discovered (99). Such degradation was more extensive in an LPS mutant (WS 1) that exhibited hypersusceptibility to the lethal action of cathepsin G. More recent work with enzymatically inactive cathepsin G showed that antibody to cathepsin G coimmunoprecipitated cathepsin G, PI (serovar 1A1), PIII, and a 45-kilodalton OMP. This interaction between cathepsin G and gonococcal OMPs is also enhanced in the LPS mutant. However, recent studies (W. M. Shafer and V. C. Onunka, unpublished observations) show that loss of PIII by insertional mutagenesis (8) does not influence the level of gonococcal susceptibility to cathepsin G.

Although binding of cathepsin G or other antimicrobial proteins to the microbial surface is a necessary step in the antimicrobial process, it is probably not sufficient for killing. A postbinding event(s) leading to the death of the target microorganism is required. The nature of the event remains to be determined. Nevertheless, some progress has been made in determining the mechanism by which gonococci succumb to antimicrobial proteins. Gonococci incubated in the presence of lethal amounts of unfractionated granule extracts continue protein and nucleic acid synthesis (14) and retain respiratory activity, but are impaired in PG synthesis (88) and show a limited capacity to divide (89). This was evidenced by a reduced incorporation of radiolabeled glucosamine into PG during incubation of gonococci with granule extracts. Such gonococci exhibit altered septa, and their OMs have an amorphous appearance. Further evidence for an involvement of PG in the lethal mechanism of antibacterial proteins (including cathepsin G) is that a mutation (*penA2*) (22, 28, 105) known to modify (28) penicillin-binding protein 2 (PBP-2) (a transglycosylase-transpeptidase) involved in the terminal stages of PG synthesis (105) renders gonococci hypersusceptible to both crude granule extracts

(22) and cathepsin G (98–101). Hence, cathepsin G may exert its antigonococcal activity against gonococci by binding to the surface, thereby causing damage to the OM, which then permits interference with enzymes (such as PBP-2) involved in the terminal stages of PG synthesis. How does a mutation that decreases the binding of penicillin to PBP-2 increase susceptibility to cathepsin G? It appears that granule proteins inhibit the binding of radiolabeled penicillin to PBPs, suggesting that granule proteins such as cathepsin G may have significant affinity for PBPs (R. F. Rest, unpublished data). Thus, mutations that alter PBPs may enhance the binding of cathepsin G, thereby leading to an inhibition of PG synthesis.

Recently, an 870-base-pair complementary deoxyribonucleic acid sequence having the coding capacity for a full-length molecule of cathepsin G has been isolated (93), cloned into λ gt11, and subcloned behind a T7 ribonucleic acid polymerase promoter in pT7-6 (W. M. Shafer, G. Salvesen, and J. Travis, submitted for publication). With this plasmid expression system, recombinant cathepsin G has been produced in *Escherichia coli*. The isolated recombinant polypeptide has bactericidal activity against gonococci that is identical to that of the lysosomal cathepsin G. The fact that recombinant cathepsin G kills gonococci (and other cathepsin G-susceptible bacteria) indicates that the antibacterial domain(s) of cathepsin G resides within its primary structure. It should now be possible, through recombinant deoxyribonucleic acid and mutagenic techniques, to identify such "antimicrobial" domains of cathepsin G. This will be of great benefit in determining the mechanism of killing.

Degradation of Gonococci by Lysosomal Enzymes

Degradation of gonococcal antigens is of importance since surface antigens or potentially toxic substances (LPS and PG) may be altered. Such action could modify antigenicity or biologic activity. Only a few studies to date, however, have examined this topic.

Proteases (notably the neutral serine proteases elastase and cathepsin G) degrade a number of gonococcal OMPs. PIB and PIIs are susceptible to PMN elastase (30, 85). This occurs in vitro when highly purified elastase and gonococci or OM preparations are used and in situ when gonococci are present in phagolysosomes. Elastase also has the capacity to cleave highly purified pili with at least one cleavage site in or near the highly conserved CNBR-2 domain of pilin (106). Such cleavage of pili in antigen-presenting macrophages may alter the domains of pilin thought to be important in biologic and immunologic activity. Cathepsin G is a much weaker protease than elastase, but it can degrade PIII and the major iron-regulated OMP termed MIRP 37 (99). The PIA molecule appears to be somewhat resistant to cathepsin G, but the PIB subclass can be degraded (Shafer et al., unpublished). Other hydrolyses in granules may also participate in digestion of the gonococcal cell envelope. Certainly lysozyme (present in both specific and azurophil granules [104]) may have a role in degrading gonococcal PG in phagolysosomes. An unrelated lysosomal enzyme has been described by Striker et al. (108) that is activated by acidic conditions and appears to remove terminal glucosamine residues in PG. Since this enzyme is activated by acidic conditions, it probably performs a PG-modifying activity in the terminal stages of phagolysosome development when the environment is acidic. The number of lysosomal hydrolyses that can attack substrates like PG (108) may give rise to diverse types of PG fragments that are thought to be liberated in vivo and may regulate human

physiology. In this respect, recent studies by Powers and Shafer (unpublished observations) indicate that cathepsin G can cleave PG fragments of strain RD5 liberated after digestion with lysozyme. Cathepsin G cleavage of PG was predicted, since molecular modeling of the active site of cathepsin G revealed that diaminopimelic acid fits into the active site.

What is the fate of the gonococcal antigens liberated from digested gonococci or modified after the antigens themselves have been degraded? Is their presence in tissues and/or fluids significant for the pathology of the different forms of gonorrhea? Do they have immunomodulatory activities? For instance, both gonococcal PG and LPS have toxic activities when prepared from bacteria grown under laboratory conditions. What are their activities in vivo after being processed by phagocytic cells? Answers to these questions are not available. However, this should be a fertile area of research in years to come, particularly since more information is becoming available regarding the structure of gonococcal LPS and PG, as well as the PMN hydrolyses that modify their structure.

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LITERATURE CITED

1. Bessen, D., and E. C. Gotschlich. 1986. Interactions of gonococci with HeLa cells: attachment, detachment, replication, penetration, and the role of protein II. *Infect. Immun.* **54**: 154–160.
2. Bessen, D., and E. C. Gotschlich. 1987. Chemical characterization of binding properties of opacity-associate protein II from *Neisseria gonorrhoeae*. *Infect. Immun.* **55**:141–147.
3. Black, J., R. S. Schwalbe, I. Nachamkin, and J. G. Cannon. 1984. Characterization of *Neisseria gonorrhoeae* protein II phase variation by use of monoclonal antibodies. *Infect. Immun.* **45**:453–457.
4. Black, J. R., W. J. Black, and J. G. Cannon. 1985. Neisserial antigen H.8 is immunogenic in patients with disseminated gonococcal and meningococcal infections. *J. Infect. Dis.* **151**: 650–657.
5. Blake, M. S. 1985. Functions of the outer membrane proteins of *Neisseria gonorrhoeae*, p. 51–66. *In* G. G. Jackson and H. Thomas (ed.), *The pathogenesis of bacterial infections*. Springer-Verlag KG, Berlin.
6. Blake, M. S., and E. C. Gotschlich. 1983. Gonococcal membrane proteins: speculations on their role in pathogenesis. *Prog. Allergy* **33**:298–313.
7. Blake, M. S., and E. C. Gotschlich. 1987. Functional and immunologic properties of pathogenic *Neisseria* surface proteins, p. 377–400. *In* M. Inouye (ed.), *Bacterial outer membranes as model systems*. John Wiley & Sons, Inc., New York.
8. Blake, M. S., L. M. Wetzler, E. C. Gotschlich, and P. A. Rice. 1989. Protein III: structure, function, and genetics. *Clin. Microbiol. Rev.* **2**(Suppl.):S60–S63.
9. Britigan, B. E., and M. S. Cohen. 1985. Effect of growth in serum on uptake of *Neisseria gonorrhoeae* by human neutrophils. *J. Infect. Dis.* **152**:330–338.
10. Britigan, B. E., and M. S. Cohen. 1986. Effects of human serum on bacterial competition with neutrophils for molecular oxygen. *Infect. Immun.* **52**:657–663.
11. Britigan, B. E., D. Klapper, T. Svendsen, and M. S. Cohen. 1988. Phagocyte-derived lactate stimulates oxygen consumption by *Neisseria gonorrhoeae*. An unrecognized aspect of the oxygen metabolism of phagocytes. *J. Clin. Invest.* **81**:318–324.

12. Brooks, G. F., K. S. Israel, and B. H. Petersen. 1976. Bactericidal and opsonic activity against *Neisseria gonorrhoeae* in sera from patients with disseminated gonococcal infection. *J. Infect. Dis.* 134:450-462.
13. Buchanan, T. M., and E. A. Pearce. 1976. Pili as a mediator of the attachment of gonococci to human erythrocytes. *Infect. Immun.* 13:1483-1489.
14. Buck, P., and R. F. Rest. 1981. Effects of human neutrophil granule extracts on macromolecular synthesis in *Neisseria gonorrhoeae*. *Infect. Immun.* 33:426-433.
15. Casey, S. G., W. M. Shafer, and J. K. Spitznagel. 1985. Anaerobiosis increases resistance of *Neisseria gonorrhoeae* to oxygen-independent antimicrobial proteins from human polymorphonuclear granulocytes. *Infect. Immun.* 47:40-47.
16. Casey, S. G., W. M. Shafer, and J. K. Spitznagel. 1986. *Neisseria gonorrhoeae* survives intraleukocytic oxygen-independent antimicrobial capacities of anaerobic and aerobic granulocytes in the presence of pyocin lethal for extracellular gonococci. *Infect. Immun.* 52:384-389.
17. Casey, S. G., D. R. Veale, and H. Smith. 1979. Demonstration of intracellular growth of gonococci in human phagocytes using spectinomycin to kill extracellular organisms. *J. Gen. Microbiol.* 113:395-398.
18. Casey, S. G., D. R. Veale, and H. Smith. 1980. Intracellular survival of *Neisseria gonorrhoeae* in human urethral exudate. *FEMS Microbiol. Lett.* 8:97-100.
19. Casey, S. G., D. R. Veale, and H. Smith. 1983. Cytotoxicity of *Neisseria gonorrhoeae* for human peripheral blood phagocytes. *J. Gen. Microbiol.* 129:1097-1102.
20. Connell, T. D., W. L. Black, T. H. Kawula, D. S. Barritt, J. F. Dempsey, K. Kverland, A. Stephenson, B. S. Schepart, G. L. Murphy, and J. G. Cannon. 1988. Recombination among protein II genes of *Neisseria gonorrhoeae* generates new coding sequences and increases structural variability in the protein II family. *Mol. Microbiol.* 2:227-238.
21. Cooper, M. D., P. A. McGraw, and M. A. Melly. 1986. Localization of gonococcal lipopolysaccharide and its relationship to toxic damage in human fallopian tube mucosa. *Infect. Immun.* 51:425-430.
22. Daly, J. A., T. J. Lee, J. K. Spitznagel, and P. F. Sparling. 1982. Gonococci with mutations to low-level penicillin resistance exhibit increased sensitivity to the oxygen-independent bactericidal activity of human polymorphonuclear leukocyte granule extracts. *Infect. Immun.* 35:826-833.
23. Demarco de Hormaeché, R., H. Jessop, and K. Senior. 1988. Gonococcal variants selected by growth in vivo or in vitro have antigenically different LPS. *Microb. Pathog.* 4:289-287.
24. Densen, P., S. Gulati, and P. A. Rice. 1987. Specificity of antibodies against *Neisseria gonorrhoeae* that stimulate neutrophil chemotaxis: role of antibodies directed against lipooligosaccharides. *J. Clin. Invest.* 80:78-87.
25. Densen, P., and G. L. Mandell. 1978. Gonococcal interactions with polymorphonuclear neutrophils. Importance of the phagosome. *J. Clin. Invest.* 62:1161-1171.
26. Diaz, J. A., and J. E. Heckels. 1984. Antigenic variation of outer membrane PII in colonial variants of *Neisseria gonorrhoeae* P9. *J. Gen. Microbiol.* 128:585-591.
27. Dilworth, J. A., J. O. Hendley, and G. L. Mandell. 1975. Attachment and ingestion of gonococci by human neutrophils. *Infect. Immun.* 11:512-516.
28. Dougherty, T. J., A. E. Koller, and A. Tomasz. 1980. Penicillin-binding proteins of penicillin-susceptible and intrinsically resistant *Neisseria gonorrhoeae*. *Antimicrob. Agents Chemother.* 18:730-737.
29. Easman, V., E. P. Noble, and R. L. Stjernholm. 1965. Carbohydrate metabolism in leukocytes. The metabolism of mannose and fructose. *Acta Chem. Scand.* 19:1672-1679.
30. Eaton, L. J., and R. F. Rest. 1983. In vivo degradation of gonococcal outer membrane proteins within human leukocyte phagolysosomes. *Infect. Immun.* 42:1034-1040.
31. Evans, B. 1977. Ultrastructural study of cervical gonorrhea. *Br. J. Vener. Dis.* 24:137-147.
32. Fancioli, P., H. Shio, R. B. Roberts, and M. Muller. 1983. Phagocytosis and killing of *Neisseria gonorrhoeae* by *Trichomonas vaginalis*. *J. Infect. Dis.* 147:87-93.
33. Fischer, S. H., and R. F. Rest. 1988. Gonococci possessing only specific P.II outer membrane proteins stimulate and adhere to human neutrophils. *Infect. Immun.* 56:1574-1579.
34. Fohn, M. J., T. A. Mietzner, T. W. Hubbard, S. A. Morse, and E. W. Hook. 1987. Human immunoglobulin G antibody response to the major iron-regulated protein. *Infect. Immun.* 55:3065-3069.
35. Fontaine, E. A., D. Taylor-Robinson, N. F. Hanna, E. D. Hanna, and E. D. Coufalik. 1982. Anaerobes in men with urethritis. *Br. J. Vener. Dis.* 58:321-326.
36. Fukuda, M., S. R. Carlsson, J. C. Klock, and A. Dell. 1986. Structure of O-linked oligosaccharides isolated from normal granulocytes, CML cells, and AML cells. *J. Biol. Chem.* 261:12796-12806.
37. Fukuda, M. N., A. Dell, J. E. Oates, J. C. Klock, and M. Fukuda. 1985. Structure of glycosphingolipids isolated from human granulocytes. The presence of a series of linear poly-N-acetyllactosaminylceramide and its significance in glycolipids of whole blood cells. *J. Biol. Chem.* 260:1067-1082.
38. Ganz, T., M. E. Selsted, D. Szklarek, S. S. L. Harwig, K. Daher, D. F. Bainton, and R. I. Lehrer. 1985. Defensins: natural peptide antibiotics of human neutrophils. *J. Clin. Invest.* 76:1427-1434.
39. Geoghegan, K. F., J. L. Dallas, and R. E. Feeney. 1980. Periodate inactivation of ovotransferrin and human serum transferrin. *J. Biol. Chem.* 255:11429-11434.
40. Gibbs, C., R. Hass, and T. F. Meyer. 1988. Structural and functional modulation of gonococcal surface proteins. *Microb. Pathog.* 4:393-399.
41. Gibbs, D. L., and R. B. Roberts. 1975. The interaction *in vitro* between human polymorphonuclear leukocytes and *Neisseria gonorrhoeae* cultivated in the chick embryo. *J. Exp. Med.* 141:155-171.
42. Gubish, E. R., K. C. S. Chen, and T. M. Buchanan. 1982. Attachment of gonococcal pili to lectin-resistant clone of Chinese hamster ovary cells. *Infect. Immun.* 37:189-194.
43. Haines, K. A., L. Yeh, M. S. Blake, P. Cristello, H. Korchak, and G. Weissmann. 1988. Protein I, a translocatable ion channel from *Neisseria gonorrhoeae*, selectively inhibits exocytosis from human neutrophils without inhibiting O₂-generation. *J. Biol. Chem.* 263:945-951.
44. Hansen, M. V., and C. E. Wilde. 1984. Conservation of peptide structure of outer membrane protein macromolecular complex from *Neisseria gonorrhoeae*. *Infect. Immun.* 43:839-845.
45. Heckels, J. E. 1977. Structural comparison of *Neisseria gonorrhoeae* outer membrane proteins. *J. Bacteriol.* 125:736-742.
46. Heckels, J. E. 1984. Molecular studies on the pathogenesis of gonorrhea. *J. Med. Microbiol.* 18:293-307.
47. Ison, C. A. 1986. The specificity of serum and local antibodies in female gonorrhea. *Clin. Exp. Immunol.* 65:199-206.
48. James, J. F., C. J. Lammel, D. L. Draper, D. A. Brown, R. L. Sweet, and G. F. Brooks. 1983. Gonococcal attachment to eukaryotic cells. *Sex. Transm. Dis.* 10:173-179.
49. James-Holmquest, A. N., R. D. Wende, R. L. Mudd, and R. P. Williams. 1973. Comparisons of atmospheric conditions for culture of clinical specimens of *Neisseria gonorrhoeae*. *Appl. Microbiol.* 26:466-469.
50. Kellogg, D. S., J. A. Crawford, and C. S. Callaway. 1983. Cultivation of *Neisseria gonorrhoeae* under low-oxygen conditions. *J. Clin. Microbiol.* 18:178-184.
51. Kellogg, D. S. J., I. R. Cohen, A. C. Norins, A. L. Schroeter, and G. Reising. 1968. *Neisseria gonorrhoeae*. II. Colonial variation and pathogenicity during 35 months in vitro. *J. Bacteriol.* 96:596-605.
52. Kellogg, D. S. J., W. L. J. Peacock, W. E. Deacon, L. Brown, and C. I. Pirkle. 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. *J. Bacteriol.* 85:1274-1279.
53. Kinane, D. F., D. M. Weir, C. C. Blackwell, and F. P. Winstanley. 1984. Binding of *Neisseria gonorrhoeae* by lectin-like receptors on human phagocytes. *J. Clin. Lab. Immunol.*

- 13:107-110.
54. King, G., and J. Swanson. 1978. Studies on gonococcus infections. XV. Identification of surface proteins of *Neisseria gonorrhoeae* correlated with leukocyte association. *Infect. Immun.* **21**:575-584.
55. Klimpel, K. W., and W. L. Clark. 1988. Multiple protein differences exist between *Neisseria gonorrhoeae* type 1 and type 4. *Infect. Immun.* **56**:808-814.
56. Knapp, J. S., and V. L. Clark. 1983. Anaerobic growth of *Neisseria gonorrhoeae* coupled to nitrite reduction. *Infect. Immun.* **46**:176-181.
57. Koransky, J. R., R. W. Scales, and S. J. Kraus. 1975. Bacterial hemagglutination by *Neisseria gonorrhoeae*. *Infect. Immun.* **12**:495-498.
58. Krieger, A. G., N. L. Schiller, and R. A. Roberts. 1980. Gonococci-human polymorphonuclear leukocyte interactions: metabolic studies associated with attachment and ingestion. *Infect. Immun.* **28**:991-1000.
59. Lambden, P. R., and J. E. Heckels. 1979. Outer membrane protein composition and colonial morphology of *Neisseria gonorrhoeae* strain P9. *FEMS Microbiol. Lett.* **5**:263-265.
60. Lambden, P. R., J. E. Heckels, L. T. James, and P. J. Watt. 1979. Variations in surface protein composition of *Neisseria gonorrhoeae* associated with virulence properties. *J. Gen. Microbiol.* **114**:305-312.
61. Lambden, P. R., J. N. Robertson, and P. J. Watt. 1980. Biological properties of two distinct pilus types produced by isogenic variants of *Neisseria gonorrhoeae* P9. *J. Bacteriol.* **141**:393-396.
62. Lambden, P. R., J. N. Robertson, and P. J. Watt. 1981. The preparation and properties of alpha and beta pili from variants of *Neisseria gonorrhoeae* P9. *J. Gen. Microbiol.* **124**:109-117.
63. Lammel, C. J., R. L. Sweet, P. A. Rice, J. S. Knapp, G. Schoolnik, D. C. Heilbron, and G. F. Brooks. 1985. Antibody-antigen specificity in the immune response to interaction with *Neisseria gonorrhoeae*. *J. Infect. Dis.* **152**:990-999.
64. Lynch, E. C., M. S. Blake, E. C. Gotschlich, and A. Mauro. 1984. Studies of porins spontaneously transferred from whole cells and reconstituted from purified proteins of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Biophys. J.* **45**:104-107.
65. McMillan, A., G. McNeillage, and H. Young. 1979. Antibodies to *Neisseria gonorrhoeae*: a study of the urethral exudates of 232 men. *J. Infect. Dis.* **140**:89-95.
66. Metchnikoff, E. 1905. *Immunity in Infective Diseases*. Cambridge University Press, Cambridge.
67. Muir, L. L., R. A. Strugnell, and J. K. Davis. 1988. Proteins that appear to be associated with pili in *Neisseria gonorrhoeae*. *Infect. Immun.* **56**:1743-1747.
68. Novotony, P., J. A. Short, and P. D. Walker. 1975. An electron-microscope study of naturally occurring and cultured cells of *Neisseria gonorrhoeae*. *J. Med. Microbiol.* **8**:413-427.
69. Odeberg, H., and I. Olsson. 1975. Antibacterial activity of cationic proteins from human granulocytes. *J. Clin. Invest.* **56**:1118-1124.
70. Odeberg, H., and I. Olsson. 1976. Mechanisms for the microbicidal activity of cationic proteins from human granulocytes. *Infect. Immun.* **14**:1269-1275.
71. Ofek, I., E. H. Beachey, and A. L. Bisno. 1974. Resistance of *Neisseria gonorrhoeae* to phagocytosis: relationship to colonial morphology and surface pili. *J. Infect. Dis.* **129**:310-316.
72. Ovcinnikov, N. M., and V. V. Delectoskij. 1971. Electron microscope studies of gonococci in the urethral secretions of patients with gonorrhea. *Br. J. Vener. Dis.* **47**:419-439.
73. Parsons, N. J., A. A. Kwaasi, P. V. Patel, P. M. V. Martin, and H. Smith. 1985. Association of resistance of *Neisseria gonorrhoeae* to killing by human phagocytes with outer membrane proteins of about 20 kilodaltons. *J. Gen. Microbiol.* **131**:601-610.
74. Parsons, N. J., A. A. Kwaasi, P. V. Patel, C. A. Nairn, and H. Smith. 1986. A determinant of resistance of *Neisseria gonorrhoeae* to killing by human phagocytes: an outer membrane lipoprotein of about 20 kDa with a high content of glutamic acid. *J. Gen. Microbiol.* **132**:3277-3287.
75. Parsons, N. J., A. A. Kwaasi, V. Y. Perera, P. V. Patel, P. M. Martin, and H. Smith. 1982. Outer membrane proteins of *Neisseria gonorrhoeae* associated with survival within human polymorphonuclear phagocytes. *J. Gen. Microbiol.* **128**:3077-3081.
76. Parsons, N. J., A. A. Kwaasi, J. A. Turner, D. R. Veale, V. Y. Perera, C. W. Penn, and H. Smith. 1981. Investigation of the determinants of the survival of *Neisseria gonorrhoeae* within human polymorphonuclear phagocytes. *J. Gen. Microbiol.* **127**:103-112.
77. Pearce, W. A., and T. M. Buchanan. 1980. Structure and cell membrane-binding properties of bacterial fimbriae, p. 291-344. In E. H. Beachey (ed.), *Bacterial adherence*. Chapman & Hall, Ltd., London.
78. Perrollet, H., and R. M. F. Guinet. 1986. Gonococcal cell wall lectin-adhesin with vaccine potential. *Lancet* **i**:1269-1270.
79. Petersen, B. H., and R. S. Rosenthal. 1982. Complement consumption by gonococcal peptidoglycan. *Infect. Immun.* **35**:442-448.
80. Punsalang, A. P., and W. D. Sawyer. 1973. Role of pili in the virulence of *Neisseria gonorrhoeae*. *Infect. Immun.* **8**:255-263.
81. Rest, R. F. 1979. Killing of *Neisseria gonorrhoeae* by human polymorphonuclear granule extracts. *Infect. Immun.* **25**:574-579.
82. Rest, R. F., C. Farrell, and F. Naidu. 1988. Mannose inhibits the human polymorphonuclear neutrophil oxidative burst. *J. Leukocyte Biol.* **43**:158-164.
83. Rest, R. F., S. H. Fischer, Z. Z. Ingham, and J. F. Jones. 1982. Interactions of *Neisseria gonorrhoeae* with human neutrophils: effects of serum and gonococcal opacity on killing and chemiluminescence. *Infect. Immun.* **36**:737-744.
84. Rest, R. F., N. Lee, and C. Bowden. 1985. Stimulation of human leukocytes by P.II gonococci is mediated by lectin-like gonococcal components. *Infect. Immun.* **50**:116-122.
85. Rest, R. F., and E. Pretzer. 1981. Degradation of gonococcal outer membrane proteins by human neutrophil lysosomal proteases. *Infect. Immun.* **34**:62-68.
86. Rice, P. A., and D. L. Kasper. 1982. Characterization of serum resistance of *Neisseria gonorrhoeae* that disseminate: role of blocking antibody and gonococcal outer membrane proteins. *J. Clin. Invest.* **70**:157-167.
87. Rice, P. A., W. M. McCormack, and D. L. Kasper. 1980. Natural serum bactericidal activity against *Neisseria gonorrhoeae* isolates from disseminated, locally invasive, and uncomplicated disease. *J. Immunol.* **124**:2105-2109.
88. Rock, J. P., and R. F. Rest. 1985. Effect of human neutrophil lysosomal contents on peptidoglycan turnover and membrane structure in *Neisseria gonorrhoeae*, p. 344-351. In G. K. Schoolnik, G. F. Brooks, S. Falkow, C. E. Frasch, J. S. Knapp, J. A. McCutchan, and S. A. Morse (ed.), *The pathogenic neisseriae*. American Society for Microbiology, Washington, D.C.
89. Rock, J. P., and R. F. Rest. 1988. Damage to membranes of *Neisseria gonorrhoeae* caused by human neutrophil granule extracts. *J. Gen. Microbiol.* **134**:509-519.
90. Rosenthal, R. S., W. J. Folkening, D. R. Miller, and S. C. Swim. 1983. Resistance of O-acetylated gonococcal peptidoglycan to human peptidoglycan-degrading enzymes. *Infect. Immun.* **40**:903-911.
91. Rosenthal, R. S., R. S. Fulbright, M. E. Eads, and W. D. Sawyer. 1977. Ethylenediaminetetraacetic acid-sensitive antiphagocytic activity of *Neisseria gonorrhoeae*. *Infect. Immun.* **15**:817-827.
92. Ross, S. C., and P. Densen. 1985. Opsonophagocytosis of *Neisseria gonorrhoeae*: interaction of local and disseminated isolates with complement and neutrophils. *J. Infect. Dis.* **151**:33-41.
93. Salvesen, G., D. Farley, J. Shuman, A. Przybyla, C. Reilly, and J. Travis. 1987. Molecular cloning of human cathepsin G: structural similarity to mast cell and cytotoxic T lymphocyte proteinases. *Biochemistry* **26**:2289-2293.
94. Sarafian, S. K., M. R. Tam, and S. A. Morse. 1983. Gonococcal

- protein I-specific opsonic IgG in normal human serum. *J. Infect. Dis.* **148**:1025-1032.
95. Schiller, N. L., G. L. Friedman, and R. B. Roberts. 1979. The role of natural IgG and complement in the phagocytosis of type 4 *Neisseria gonorrhoeae* by human polymorphonuclear leukocytes. *J. Infect. Dis.* **140**:698-707.
 96. Schneider, H., C. A. Hammack, M. A. Apicella, and J. M. Griffiss. 1988. Instability of expression of lipooligosaccharides and their epitopes in *Neisseria gonorrhoeae*. *Infect. Immun.* **56**:942-946.
 97. Schwalbe, R. S., P. F. Sparling, and J. G. Cannon. 1985. Variation of *Neisseria gonorrhoeae* protein II among isolates from an outbreak caused by a single gonococcal strain. *Infect. Immun.* **49**:250-252.
 98. Shafer, W. M. 1988. Lipopolysaccharide masking of gonococcal outer-membrane proteins modulates binding of bactericidal cathepsin G to gonococci. *J. Gen. Microbiol.* **134**:539-545.
 99. Shafer, W. M., and S. A. Morse. 1987. Cleavage of the protein III and major iron-regulated protein of *Neisseria gonorrhoeae* by lysosomal cathepsin G. *J. Gen. Microbiol.* **133**:155-162.
 100. Shafer, W. M., V. C. Onunka, and P. J. Hitchcock. 1986. A spontaneous mutant of *Neisseria gonorrhoeae* with decreased resistance to neutrophil granule proteins. *J. Infect. Dis.* **153**:410-417.
 101. Shafer, W. M., V. C. Onunka, and L. E. Martin. 1986. Antigonococcal activity of human neutrophil cathepsin G. *Infect. Immun.* **54**:184-188.
 102. Siegel, M., D. Olsen, C. Critchlow, and T. M. Buchanan. 1982. Gonococcal pili: safety and immunogenicity in humans and antibody function in vitro. *J. Infect. Dis.* **145**:300-310.
 103. Sparling, P. F., J. G. Cannon, and M. So. 1986. Phase and antigenic variation of pili and outer membrane protein II of *Neisseria gonorrhoeae*. *J. Infect. Dis.* **153**:196-201.
 104. Spitznagel, J. K., and W. M. Shafer. 1985. Neutrophil killing of bacteria by oxygen-independent mechanisms: a historical summary. *Rev. Infect. Dis.* **7**:398-403.
 105. Spratt, B. G. 1988. Hybrid penicillin-binding proteins in penicillin-resistant *Neisseria gonorrhoeae*. *Nature (London)* **332**:173-176.
 106. Stephens, D. S., and W. M. Shafer. 1988. Cleavage of gonococcal pilin and alteration of common pilin epitopes by human neutrophil lysosomal elastase. *FEMS Microbiol. Lett.* **52**:47-52.
 107. Stern, A., M. Brown, P. Nickel, and T. F. Meyer. 1986. Opacity genes in *Neisseria gonorrhoeae*: control of phase and antigenic variation. *Cell* **47**:61-71.
 108. Striker, R., M. E. Kline, R. A. Haak, R. F. Rest, and R. S. Rosenthal. 1987. Degradation of gonococcal peptidoglycan by granule extract from human neutrophils: demonstration of *N*-acetylglucosaminidase activity that utilizes peptidoglycan substrates. *Infect. Immun.* **55**:2579-2584.
 109. Stromberg, N., C. Deal, G. Nyberg, S. Normark, M. So, and K.-A. Karlsson. 1988. Identification of carbohydrate structures that are possible receptors for *Neisseria gonorrhoeae*. *Proc. Natl. Acad. Sci. USA* **85**:4902-4906.
 110. Sugawara, R. J., J. G. Cannon, W. J. Black, I. Nachamkin, R. L. Sweet, and G. F. Brooks. 1983. Inhibition of *Neisseria gonorrhoeae* attachment to HeLa cells with monoclonal antibody directed against a protein II. *Infect. Immun.* **42**:980-985.
 111. Swanson, J. 1973. Studies on gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. *J. Exp. Med.* **137**:571-589.
 112. Swanson, J. 1978. Studies on gonococcus infection. XII. Colony color and opacity variants of gonococci. *Infect. Immun.* **19**:320-331.
 113. Swanson, J. 1978. Studies on gonococcus infection. XIV. Cell wall protein differences among colony/opacity variants of *Neisseria gonorrhoeae*. *Infect. Immun.* **21**:292-302.
 114. Swanson, J. 1980. ¹²⁵I-labeled peptide mapping of some heat-modifiable proteins of the gonococcal outer membrane. *Infect. Immun.* **28**:54-64.
 115. Swanson, J. 1982. Colony opacity and protein II composition of gonococci. *Infect. Immun.* **37**:359-368.
 116. Swanson, J. 1983. Gonococcal adherence: selected topics. *Rev. Infect. Dis.* **5**:S678-S684.
 117. Swanson, J., G. J. King, and B. Seligs. 1975. Studies on gonococcus infection. VII. In vitro killing of gonococci by human leukocytes. *Infect. Immun.* **11**:65-68.
 118. Swanson, J., K. Robbins, O. Barrera, D. Corwin, J. Boslego, J. Ciak, M. Blake, and J. M. Koomey. 1988. Gonococcal pilin variants in experimental gonorrhea. *J. Exp. Med.* **165**:1344-1357.
 119. Swanson, J., E. Sparks, D. Young, and G. King. 1975. Studies on gonococcus infection. X. Pili and leukocyte association factors as mediators of interactions between gonococci and eucaryotic cells in vitro. *Infect. Immun.* **11**:1352-1361.
 120. Swanson, J., E. Sparks, E. Zeligs, M. A. Siam, and C. Parrott. 1974. Studies on gonococcus infection. V. Observations on in vitro interactions of gonococci and human neutrophils. *Infect. Immun.* **10**:633-644.
 121. Symington, F. W., D. L. Hedges, and S. I. Hakomori. 1985. Glycolipid antigens of human PMN and the inducible HL-60 myeloid leukemia line. *J. Immunol.* **134**:2498-2506.
 122. Thomas, D. W., J. C. Hill, and F. J. Tyeryar, Jr. 1973. Interactions of gonococci with phagocytic leukocytes from men and mice. *Infect. Immun.* **8**:98-104.
 123. Thongthai, C., and W. D. Sawyer. 1973. Studies on the virulence of *Neisseria gonorrhoeae*. I. Relation of colonial morphology and resistance to phagocytosis by polymorphonuclear leukocytes. *Infect. Immun.* **7**:373-379.
 124. Tramont, E. C. 1980. Antigenic specificity of antibodies in vaginal secretions during infection with *Neisseria gonorrhoeae*. *J. Infect. Dis.* **142**:23-31.
 125. Trust, T. J., P. R. Lambden, and P. J. Watt. 1980. The cohesive properties of variants of *Neisseria gonorrhoeae* strain P9: specific pilus-mediated and non-specific interactions. *J. Gen. Microbiol.* **119**:179-187.
 126. Veale, D. R., M. Goldner, C. W. Penn, J. Ward, and H. Smith. 1979. The intracellular survival and growth of gonococci in human phagocytes. *J. Gen. Microbiol.* **113**:383-393.
 127. Veale, D. R., D. Sen, C. W. Penn, H. Finch, H. Smith, and K. Witt. 1977. Interactions of *Neisseria gonorrhoeae* with guinea pig defence mechanisms in subcutaneously implanted chambers. *FEMS Microbiol. Lett.* **1**:3-6.
 128. Virji, M., and J. S. Everson. 1981. Comparative virulence of opacity variants of *Neisseria gonorrhoeae* P9. *Infect. Immun.* **31**:965-970.
 129. Virji, M., and J. E. Heckels. 1986. The effect of protein II and pili on the interaction of *Neisseria gonorrhoeae* with human polymorphonuclear leukocytes. *J. Gen. Microbiol.* **132**:503-512.
 130. Ward, M. E., A. A. Glynn, and P. J. Watt. 1972. Fate of *Neisseria gonorrhoeae* in polymorphonuclear leukocytes: an electronmicroscopic study of the natural disease. *Br. J. Exp. Pathol.* **53**:289-294.
 131. Ward, M. E., and P. J. Watt. 1972. Adherence of *Neisseria gonorrhoeae* to urethral mucosal cells. An electron microscopic study of human gonorrhea. *J. Infect. Dis.* **126**:601-604.
 132. Waters, W. A. 1964. The oxidation of 1,2-glycols, p. 72-81. In W. A. Waters (ed.), *Mechanisms of oxidation of organic compounds*. Methuen & Co., Ltd., London.
 133. Wiseman, G. M., P. McNicol, C. J. Lian, and D. S. Primrose. 1981. Some properties of the human erythrocyte receptors for *Neisseria gonorrhoeae*. *Can. J. Microbiol.* **27**:1035-1043.
 134. Witt, K., D. R. Veale, H. Finch, C. W. Penn, D. Sen, and H. Smith. 1976. Resistance of *Neisseria gonorrhoeae* grown in vivo to ingestion and digestion by phagocytes of human blood. *J. Gen. Microbiol.* **96**:341-350.
 135. Young, J. D.-E., M. Blake, A. Mauro, and Z. A. Cohn. 1983. Properties of the major outer membrane protein from *Neisseria gonorrhoeae* incorporated into model lipid membranes. *Proc. Natl. Acad. Sci. USA* **80**:3831-3835.
 136. Zak, K., J.-L. Diaz, D. Jackson, and J. E. Heckels. 1984. Antigenic variation during infection with *Neisseria gonorrhoeae*: detection of antibodies to surface proteins in sera of patients with gonorrhea. *J. Infect. Dis.* **149**:166-174.