INFECTION AND IMMUNITY, Oct. 1986, p. 184–188
0019-9567/86/100184-05$02.00/0

Vol. 54, No. 1

Antigonococcal Activity of Human Neutrophil Cathepsin G

WILLIAM M. SHAFER,* VICTOR C. ONUNKA, AND LARRY E. MARTIN

Department of Microbiology and Immunology, School of Medicine, Emory University, Atlanta, Georgia 30322

Received 14 April 1986/Accepted 19 June 1986

We have shown that lysosomal cathepsin G, prepared from acid extracts of granules derived from human polymorphonuclear granulocytes, exhibits potent in vitro antimicrobial activity against *Neisseria gonorrhoeae*. An isolated isozyme of cathepsin G was found to exhibit antigonococcal activity by a nonenzymatic mechanism in a time-dependent manner. Moreover, we observed that the antigonococcal activity of cathepsin G was relatively independent of pH and evident over a pH range resembling that invoked for maturing phagolysosomes. Using a number of isogenic strains, we determined that certain mutations known to alter cell envelope structure rendered gonococci more susceptible to cathepsin G. This suggests that the susceptibility of gonococci to cathepsin G, and possibly other antimicrobial proteins derived from PMN granules, is genetically determined and possibly related to the structure of the gonococcal cell envelope.

Nonoxidative antimicrobial systems of human polymorphonuclear granulocytes (PMN) have been suggested to contribute significantly in the intraphagosomal killing of *Neisseria gonorrhoeae* (5, 14). Cationic antimicrobial proteins (CAPs), derived from the cytoplasmic granules of PMN, have been implicated in such killing of gonococci (3, 5, 14). To date, the number of proteins responsible for such behavior has not been clearly defined. However, recent studies in this laboratory suggest that at least three CAPs have the capacity to kill gonococci in vitro (5, 6, 17). These proteins include two CAPs with apparent molecular sizes of 37 and 57 kilodaltons (37K and 57K CAPs) and at least two 24- to 25-kilodalton proteins resembling those of the known isozymes of lysosomal cathepsin G (23).

Cathepsin G, a chymotrypsinlike serine protease consisting of three isozymes (23), was shown earlier by Odeberg and Ohlsson (12) to possess antibacterial activity against certain gram-positive and -negative bacteria. Antibacterial activity of cathepsin G was found by Odeberg and Ohlsson to be independent of serine esterase activity and was likely due to the highly cationic nature (pI of greater than 12.5) of the protein.

We recently obtained evidence (17) that lysosomal proteins resembling cathepsin G isozymes have the capacity to exhibit potent antigonococcal activity in vitro by a nonenzymatic process. Uniquely, our results suggested that cathepsin G was at least 100-fold more active against gonococci, compared with other bacteria such as *Staphylococcus aureus* or *Escherichia coli* (12). Thus, gonococci may represent a unique target in studies aimed at determining mechanisms by which cathepsin G exerts antibacterial action. We have designed experiments, therefore, to establish the antigonococcal capacity of cathepsin G.

MATERIALS AND METHODS

Procurement of human PMN and extraction of granules. Granulocyte concentrates containing >95% PMN were obtained by leukapheresis from two healthy individuals who denied any history of gonorrhea. The procedure was conducted after informed consent was obtained and was performed at the Emory University Hospital Cell Separator Laboratory. PMN thus obtained were homogenized as described by Rest et al. (13). Granules were collected by high-speed centrifugation and were extracted with 0.2 M sodium acetate (pH 4.0). Extracts were stored at 4°C before use.

Purification of lysosomal cathepsin G by Sepharose 4B-Trasylol chromatography and resolution of isozymes. Trasylol (the kind gift of J. K. Spitznagel, Emory University) was coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) as described by Baugh and Travis (1). Before application to the column, the crude granule extract was adjusted to pH 6.8 by the addition of 2 M Tris (unbuffered) and 0.5 M NaCl. Protein (75 mg) in 50 ml was applied to the column (2 by 0.5 cm). The column was washed sequentially with 50 ml of the following buffers: 50 mM Tris, 50 mM NaCl (pH 6.8); 50 mM Tris, 0.4 M NaCl (pH 6.8); 50 mM sodium phosphate, 0.4 M NaCl (pH 6.5); 50 mM sodium acetate, 0.4 M NaCl (pH 5.0); and 50 mM sodium acetate, 0.4 M NaCl (pH 4.5). Under these conditions, as shown earlier by Baugh and Travis (1), the majority of the granule proteins other than elastase and cathepsin G are not bound to the affinity column. Elastase elutes from the column at pH 5.0, while cathepsin G elutes from the column at pH 4.5 (1). The enzymatic activities of these proteases were analyzed as described by Starkey and Barrett (20, 21).

The cathepsin G-containing fractions were pooled and dialyzed overnight against 0.5 M NaCl, 50 mM sodium acetate pH 5.5 at 4°C. Protein (2 mg) was then applied to a carboxymethyl cellulose column (10 by 1 cm), equilibrated previously in the above buffer. The column was washed with 50 ml of buffer, after which protein that remained bound to the column was eluted with a linear salt gradient (400 ml) consisting of 0.5 to 1.0 M NaCl in 50 mM sodium acetate, pH 5.5. After the gradient had run its course, the column was washed with 50 ml of 1.5 M NaCl in 50 mM sodium acetate at pH 5.5. Protein was monitored at 280 nm, while salt concentrations were determined by conductivity measurements. Protein concentrations were determined as described by Bradford (2), with chicken egg white lysozyme as a standard.

SDS-PAGE. We used sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to evaluate the electrophoretic characteristics of the preparations of cathepsin G. SDS-PAGE was conducted as described by Laemmli (11) under reducing conditions. SDS-PAGE profiles were analyzed by silver staining (16).

* Corresponding author.

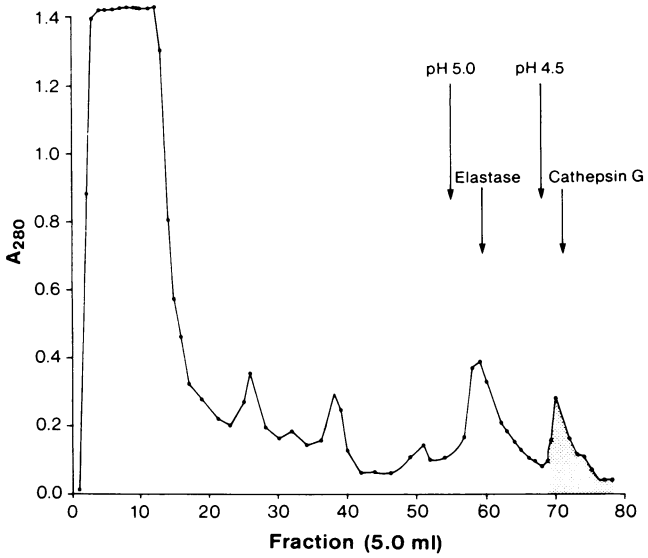

FIG. 1. Preparation of lysosomal cathepsin G by Sepharose-Trasylol affinity chromatography of crude granule extracts prepared from human PMN. Protein (75 mg) in 0.2 M sodium acetate–0.5 M NaCl (pH 6.8) was applied to the affinity column. Elastase and cathepsin G were released sequentially from the column after application of 50 mM sodium acetate–0.4 M NaCl (pH 5.0) and 50 mM sodium acetate–0.4 NaCl (pH 4.5). The darkened area highlights cathepsin G.

Bactericidal assays. *N. gonorrhoeae* FA102 was the primary test strain used. In some experiments, where indicated, isogenic variants of this strain were also studied. Gonococci were maintained as nonpiliated, transparent variants on GCB agar (Difco Laboratories, Detroit, Mich.) plates. For bactericidal assays, gonococci were scraped from an 18-h GCB agar-grown culture and suspended in 25 ml of GCB broth containing defined supplements I and II (7) and 0.43% sodium bicarbonate (wt/vol). Cultures were grown at 37°C with shaking until the A_{550} = 0.3. Before incorporation into bactericidal assays, cultures were diluted in broth to afford a cell density of ca. 10,000 CFU/ml. Antimicrobial granule proteins were dialyzed against distilled water overnight at 4°C before use. The bactericidal assay was conducted in GCB broth with sterile microtiter titer trays as described by Casey et al. (5). After 45 min of incubation at 37°C under 5% CO_2, samples were plated onto GCB agar plates. Controls consisted of gonococci incubated in the absence of granule protein. All plates were incubated for 48 h at 37°C under 5% CO_2. The percentage of gonococci surviving the bactericidal activity of the granule protein preparations was calculated by using the control cultures incubated under identical conditions.

RESULTS

Purification of cathepsin G. Lysosomal cathepsin G was obtained in partially purified form after Sepharose-Trasylol chromatography of a crude granule extract. As shown earlier by Baugh and Travis (1), cathepsin G was recovered from the affinity column after addition of the pH 4.5 buffer (Fig. 1). The protein distribution of this preparation was evaluated by silver staining of SDS-PAGE profiles. This preparation of cathepsin G consisted of two intense bands at 24 and 25.5 kilodaltons and one less intense band at ca. 28 kilodaltons (Fig. 2). The recovery of total enzymatic units of cathepsin G applied to the column was 88%, and the specific activity was increased 26-fold; the specific enzymatic activity of the crude extract was ca. 1.79 nKat (units of activity hydrolyzing 1 nmol of substrate per s per mg of protein) per mg, while that of the cathepsin G preparation obtained by affinity chromatography was 45 nKat/mg. This partially purified preparation of cathepsin G exhibited potent antigonococcal activity (the 50% effective dose [ED_{50}] was ca. 0.5 μg of protein per ml) against strain FA102, and it was at least 50-fold more active than that of the crude extract (data not shown).

Further purification and resolution of the three known isozymes (23) was achieved by ion-exchange chromatography. The resulting chromatogram (Fig. 3) showed elution of substantial protein before the application of the salt gradient. This protein was found to consist of lysosomal elastase (data not shown) with a specific activity of 110 nKat/mg. The contaminating elastase was well separated from the two other peaks that emerged from the column during the salt gradient. The first peak to emerge from the column during the gradient did so in nearly gaussian fashion, while the second, more predominant peak failed to do so. The pronounced "shoulder" of the leading edge of the second peak was similar to what Travis et al. (23) obtained in their studies

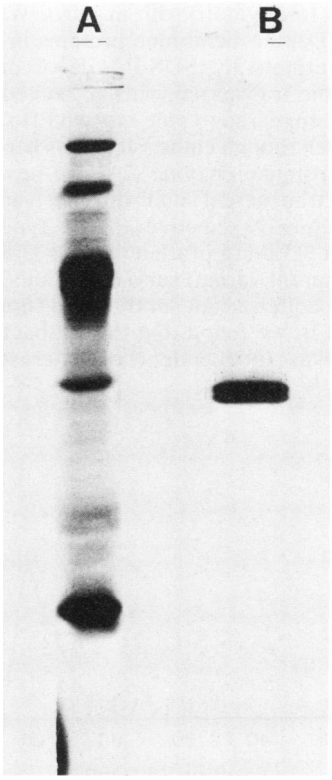

FIG. 2. SDS-PAGE profile of cathepsin G recovered from the Sepharose-Trasylol affinity column. One microgram of protein was solubilized in 0.125 Tris hydrochloride–2% (wt/vol) SDS–1% (vol/vol) beta-mercaptoethanol at 100°C for 5 min. Shown is the silver-stained profile of the resulting electropherogram. Bio-Rad protein molecular standards are presented to the left of the cathepsin G preparation. Analysis of the cathepsin G preparation resolved two major proteins at ca. 24 and 25 kilodaltons and a less intense band at ca. 28 kilodaltons. The 28-kilodalton species comigrated with preparations of elastase obtained by Sepharose-Trasylol chromatography.

dealing with the identification of the isozymes of cathepsin G. They found that two isozymes of cathepsin G eluted from their carboxymethyl cellulose column in this area, while the third isozyme was present in the preceding peak. We pooled column fractions (as shown) and tested for enzymatic and antigonococcal activity; the pooled fractions were arbitrarily referred to as isozymes I, II, and III, based on the similarity between our data and that obtained by Travis et al. (23). Each preparation was tested for antigonococcal activity at 0.5 μg of protein per ml. The results (Table 1) showed that all three preparations exhibited potent enzymatic and antigonococcal activity; the type I and II isozymes appeared to be more antimicrobial than the type III isozyme. At substantially higher concentrations of cathepsin G (15 and 10 μg/ml), less than 5% of the exposed gonococci survived (data not presented). It should be noted that the elastase preparation recovered from the column failed to kill strain FA102 in this assay (data not presented).

Antigonococcal activity of an isolated isozyme of cathepsin G. Several experiments conducted to characterize the antigonococcal activity of cathepsin G were performed with the isolated type I isozyme. We first tested whether isogenic strains of FA102 differing in colony opacity also differed in levels of susceptibility to this isozyme. We performed this analysis because Swanson (22) demonstrated that the PII outer membrane proteins thought to be responsible for colony opacity (24) rendered opaque variants of gonococci more sensitive to chymotrypsin in vitro. We tested for the presence of PII outer membrane proteins in the transparent and opaque variants by SDS-PAGE of outer membrane vesicles (10); the transparent variant lacked PIIs while the opaque variant appeared to possess two PII species (data not presented). Even though cathepsin G has been characterized as a chymotrypsinlike enzyme (23), we nevertheless found that isogenic transparent and opaque variants of strain FA102 were equally sensitive to the type I isozyme of cathepsin G; at 0.5 μg of protein per ml, 33.5% (± 3.65, n = 4) of the transparent variant survived, while 34.05% (± 1.77, n = 4) of the opaque variant survived. In support of Odeberg and Ohlsson (12), we found that the antibacterial activity of cathepsin G was independent of esterase activity. We reached this conclusion since pretreatment of the type I isozyme with diisopropylfluorophosphate readily inhibited enzymatic activity, but had no effect on antigonococcal activity (data not presented).

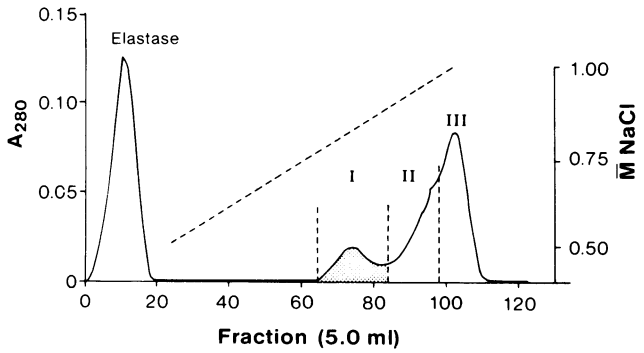

FIG. 3. Partial resolution of cathepsin G isozymes after ion-exchange chromatography. Partially purified cathepsin G recovered from the Sepharose-Trasylol affinity column was applied to a carboxymethyl cellulose column. Application of the salt gradient (see the text) resulted in partial separation of the three known isozymes (see reference 23) rendered free of elastase. The dotted line extending from fractions 25 to 105 is the approximate molarity of NaCl. The hatched area (fractions 65 to 85) represents the type I isozyme (ca. 10% of the total cathepsin G recovered) employed in subsequent antigonococcal assays.

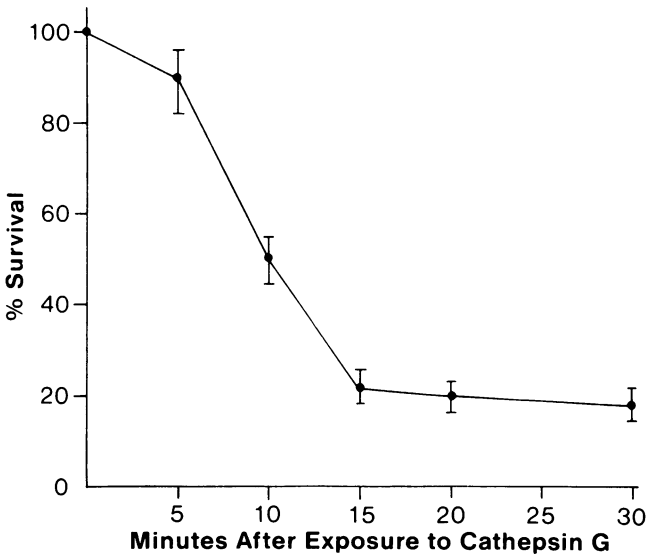

FIG. 4. Kinetics of cathepsin G mediated killing of strain FA 102. Strain FA 102 was expanded to 0.5 μg of the type I cathepsin G isozyme per ml at 37°C under 5% CO_2. Samples were taken at the indicated times. Each data point represents average values (± standard error) from three separate experiments.

The time course of killing of strain FA102 (transparent variant) by the type I isozyme was examined. We found that little or no killing occurred during the first 5 min of incubation of gonococci with the enzyme (0.5 μg/ml), but extensive killing was evident at later times (Fig. 4). When these data were plotted on a semilogarithmic scale, cathepsin G-mediated killing of gonococci resembled second-order kinetics, suggesting that two "hits" may be required to eliminate 1 CFU arising from a diplococcus (data not presented). We also found that substantial killing occurred over the pH range where the environment of the phagolysosome is thought to extend after engulfment of bacteria (15). Thus, we found (Fig. 5) that gonococci were killed readily by the type I isozyme over a pH range of 6.0 to 7.2.

Mutations that increase gonococcal resistance to penicillin increase susceptibility to cathepsin G. Earlier studies by Daly et al. (7) showed that gonococci bearing mutations that increase resistance to penicillin exhibited increased susceptibility (compared to the penicillin-sensitive parental strain) to crude extracts of azurophil granules of human PMN. Since cathepsin G is known to be localized within the azurophil granules (19), we sought to determine whether such mutations would also increase susceptibility to the type

TABLE 1. Antigonococcal activity of cathepsin G isozymes

Isozyme	% Survival[a] ± SEM (n = 4)	Enzymatic activity[b]
I	28 ± 3.6	48
II	30 ± 4.4	47
III	48 ± 2.6	47

[a] Expressed as percentage of bacteria (strain FA102) surviving in the bactericidal assay, which employed 0.5 μg of each isozyme per ml.
[b] Expressed as nKats per milligram of protein.

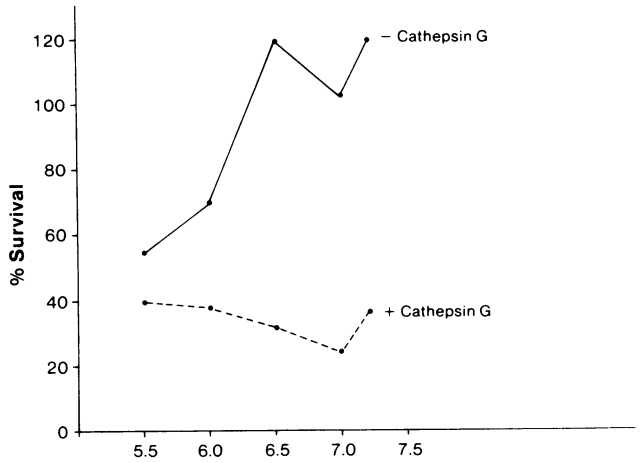

FIG. 5. The antigonococcal activity of the type I isozyme of cathepsin G extends over the proposed pH range of the maturing PMN phagolysosome (14). Strain FA102 was incubated for 30 min in the absence (●——●) or presence (●– – –●) of 0.5 μg of the type I cathepsin G per ml in GCB broth (pH 5.5 to 7.2). The results are average values from three separate experiments. For each data point, the standard error was never greater than 5%. In this experiment, it was necessary to calculate the percentage of survival relative to the input of gonococci, since the lower pH values (5.5 and 6.0) were found to be detrimental even in the absence of added cathepsin G.

I isozyme of cathepsin G. This was necessary because we have shown that one such mutation (*penA2*) increases susceptibility to 37K and 57K CAPs (6), and, as such, we questioned whether mutations such as *penA2* and an additional mutation, *mtr-2*, would universally increase gonococcal susceptibility to granule proteins. Accordingly, we examined the susceptibility to the type I isozyme of strains FA19 (the parental strain of FA102), FA102 (FA19 *penA2*), FA171 (FA19 *mtr-2*), FA136 (FA 19 *penA2* and *mtr-2*), and WSI. Strain WSI is a spontaneous mutant of FA102 (17). It is known to exhibit increased susceptibility (compared with FA102) to extracts of PMN granules and partially purified preparations of cathepsin G (17). We found that both the *penA* and *mtr-2* mutations, either individually (strains FA102 and FA171) or together (strain FA136), increased susceptibility of gonococci to the type I isozyme, compared with the wild-type parental strain FA19 (Table 2). Consistent with our earlier study (17), strain WSI was found to exhibit remarkable hypersensitivity to the type I isozyme.

TABLE 2. Gonococci with mutations which increase resistance to penicillin exhibit decreased resistance to cathepsin G

Strain	Phenotype[a]	Approx ED_{50} of cathepsin G (μg/ml)[b]
FA19	Wild type	1.0
FA102	As FA19 but *penA2*	0.125
FA171	As FA19 but *mtr-2*	0.125
FA136	As FA19 but *penA2 mtr-2*	0.2
WS1	LPS mutant of FA102	0.025

[a] Strains FA102, FA171, and FA136 were constructed by Sparling (9, 18). Strain WS1 is a spontaneous pyocin-resistant LPS mutant of FA102 (17).

[b] Values are expressed as averages from three separate determinations which employed several concentrations (2 to 0.006 μg/ml) of the type I isozyme of cathepsin G. The ED_{50} was calculated by analysis of plots of percent survival versus protein concentration.

DISCUSSION

We have shown that human PMN, lysosomal cathepsin G, and an isolated isozyme in particular, have the capacity to exert potent antigonococcal activity in vitro. To obtain cathepsin G in partially purified form, advantage was taken of its known affinity for the weak inhibitor Trasylol (1). This was accomplished after passing a crude granule extract over a Sepharose 4B-Trasylol affinity column. The known isozymes of cathepsin G were subsequently obtained (free of contaminating elastase) by ion-exchange chromatography according to the established procedure of Baugh and Travis (1). These preparations of cathepsin G were found to exhibit potent antigonococcal activity in vitro (Table 1).

A nonenzymatic process is presumably responsible for cathepsin G-mediated killing of gonococci, since preparations rendered inactive by diisopropylfluorophosphate remained antimicrobial. This observation is consistent with the earlier studies of Odeberg and Ohlsson (12). They found that heat-inactivated preparations of cathepsin G retained the capacity to kill *S. aureus*. They invoked the cationic nature of cathepsin G as responsible for killing. The precise mechanism by which this CAP mediates killing of gonococci is not known as yet. However, it is likely that the binding of cathepsin G to appropriate cell surface structures damages the cell envelope to such an extent as to be lethal. Using the isogenic strains described in Table 2, we are now conducting experiments designed to elucidate the mechanism by which gonococci succumb to the lethal action of cathepsin G.

The remarkable sensitivity of gonococci to cathepsin G suggests that this pathogen could serve as a unique target in studies designed to ascertain how this CAP elicits nonoxidative killing of bacteria. Opposed to the high concentrations of cathepsin G (ca. 100 μg/ml) required to demonstrate a bactericidal effect against either *S. aureus* or *E. coli* (12), we found that gonococci were sensitive to submicrogram quantities of cathepsin G. In vitro killing of gonococci by cathepsin G occurred in a time-dependent manner; maximal killing occurred after 15 min of incubation (Fig. 4).

Killing of gonococci by cathepsin G was evident over a pH range of 6.0 to 7.2 (Fig. 5). Since this pH range is similar to that of the maturing phagolysosome (15), it is likely that gonococci engulfed by PMN would be susceptible to killing by cathepsin G. This would be true for periods not only immediately following phagocytosis, where the intravacuolar pH is thought to be neutral, but during subsequent periods when the phagolysosome becomes acidified (15).

The observation that mutations such as *penA2* and *mtr-2*, which increase the resistance of gonococci to penicillin also increase susceptibility to cathepsin G, is consistent with the earlier work of Daly et al. (7). These authors found that such mutations rendered gonococci more susceptible to extracts of azurophil granules of human PMN. The work presented here confirms and extends their observation to that of an isolated protein (cathepsin G), known to be localized within the azurophil granules (19). We have also shown that *penA2* and *mtr-2* renders strain FA19 less resistant to 37K and 57K CAPs (6). Our finding that mutations such as *penA2* and *mtr-2* increase gonococcal susceptibility to CAPs may, in part, explain why gonococci isolated from the blood rarely bear similar mutations (7). Thus, the wild-type alleles would provide for increased sensitivity to penicillin yet increased resistance to CAPs. This might make gonococci more resistant to intraphagosomal killing by PMN. Accordingly, it would be advantageous for gonococci to harbor the wild-type penicillin-sensitive alleles during the course of dissem-

inated disease. Indeed, Cannon and Sparling (4) have advanced the same hypothesis in their recent review.

It is likely that alterations in cell envelope structure (4, 7–9, 22) due to mutations such as *penA2* and *mtr-2* have direct effects upon the general interaction of CAPs with the cell surface of *N. gonorrhoeae*. In view of this hypothesis, we are now examining the interaction of cathepsin G with gonococcal outer membrane proteins and lipopolysaccharide. In this regard, recent studies indicate that, at least for strain FA102, a major iron-regulated protein of 37 kilodaltons and outer membrane protein III are susceptible to cleavage by the enzymatic activity of cathepsin G (W. M. Shafer and S. A. Morse, submitted for publication). This suggests that certain exposed surface domains of outer membrane proteins have the capacity to bind cathepsin G. Thus, mutations that increase gonococcal susceptibility to CAPs such as cathepsin G could influence binding of CAPs to outer membrane proteins or other cell envelope structures such as lipopolysaccharide.

ACKNOWLEDGMENTS

We thank P. F. Sparling for providing bacterial strains, J. K. Spitznagel for the kind gift of Trasylol, and J. Travis for stimulating conversations dealing with cathepsin G. We also thank R. Vogler for his assistance in procuring human PMN concentrates. It is a pleasure to acknowledge J. Royer for skillful preparation of this manuscript.

This work was supported by Public Health Service grants AI 21150 (W.M.S.) and AI 17622 (John K. Spitznagel) from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Baugh, R. J., and J. Travis.** 1976. Human leukocyte elastase: rapid isolation and characterization. Biochemistry **15**:836–841.
2. **Bradford, M.** 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
3. **Buck, P., and R. F. Rest.** 1981. Effects of human neutrophil granule extracts on macromolecular synthesis in *Neisseria gonorrhoeae*. Infect. Immun. **33**:426–433.
4. **Cannon, J. G., and P. F. Sparling.** 1984. Genetics of *Neisseria gonorrhoeae*. Annu. Rev. Microbiol. **38**:121.
5. **Casey, S. G., W. M. Shafer, and J. K. Spitznagel.** 1985. Anaerobiosis increases resistance of *Neisseria gonorrhoeae* to O_2-independent antimicrobial proteins from human polymorphonuclear granulocytes. Infect. Immun. **47**:401–407.
6. **Casey, S. G., J. K. Spitznagel, and W. M. Shafer.** 1985. Resistance of *Neisseria gonorrhoeae* to oxygen-independent antimicrobial proteins from human polymorphonuclear granulocytes: role of anaerobiosis, p. 502–507. *In* G. K. Schoolnik, G. F. Brooks, S. Falkow, C. E. Frasch, J. S. Knapp, J. A. McCutchun, and S. A. Morse (ed.), The pathogenic *Neisseriae*: proceedings of the fourth international symposium. American Society for Microbiology, Washington, D.C.
7. **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.
8. **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.
9. **Guymon, L. F., D. L. Walstad, and P. F. Sparling.** 1978. Cell envelope alterations in antibiotic-sensitive and -resistant strains of *Neisseria gonorrhoeae*. J. Bacteriol. **136**:391–401.
10. **Heckels, J. E.** 1981. Structural comparison of *Neisseria gonorrhoeae* outer membrane proteins. J. Bacteriol. **145**:736–742.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
12. **Odeberg, H., and I. Ohlsson.** 1975. Antibacterial activity of cationic proteins from human granulocytes. J. Clin. Invest. **56**:1118–1124.
13. **Rest, R. F., M. H. Cooney, and J. K. Spitznagel.** 1977. Susceptibility of lipopolysaccharide mutants to the bactericidal action of human neutrophil lysosomal fractions. Infect. Immun. **16**:145–151.
14. **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 phagocyte killing and chemiluminescence. Infect. Immun. **36**:737–744.
15. **Segal, A. W., M. Geisow, R. Garcia, A. Harper, and R. Miller.** 1981. The respiratory burst of phagocytic cells is associated with a rise in vacuolar pH. Nature (London) **290**:406–409.
16. **Shafer, W. M., L. E. Martin, and J. K. Spitznagel.** 1984. Cationic antimicrobial proteins isolated from human neutrophil granulocytes in the presence of diisopropyl fluorophosphate. Infect. Immun. **45**:29–35.
17. **Shafer, W. M., V. C. Onunka, and P. J. Hitchcock.** 1986. A spontaneous mutant of *Neisseria gonorrhoeae* with decreased resistance to neutrophil granule protein. J. Infect. Dis. **153**: 910–917.
18. **Sparling, P. F., F. A. Sarubbi, Jr., and E. Blackman.** 1975. Inheritance of low-level resistance to penicillin, tetracycline, and chloramphenicol in *Neisseria gonorrhoeae*. J. Bacteriol. **124**:740–749.
19. **Spitznagel, J. K., F. G. Dalldorf, M. S. Leffell, J. D. Folds, I. R. H. Welsh, M. H. Cooney, and L. E. Martin.** 1974. Character of azurophil and specific granules purified from human polymorphonuclear leukocytes. Lab. Invest. **30**:774–785.
20. **Starkey, P. M., and A. J. Barrett.** 1976. Human cathepsin G: catalytic and immunological properties. Biochem. J. **155**:273–278.
21. **Starkey, P. M., and A. J. Barrett.** 1976. Human lysosomal elastase: catalytic and immunological properties. Biochem. J. **155**:265–271.
22. **Swanson, J.** 1980. Gonococcal growth inhibition and hydrolysis of outer membrane proteins by alpha-chymotrypsin, p. 19–24. *In* D. Danielsson and S. Normark (ed.), Genetics and immunobiology of pathogenic *Neisseria*. Norrlands-tryck, Umea, Sweden.
23. **Travis, T. J., P. J. Giles, L. Procelli, C. F. Reily, R. Baugh, and J. Powers.** 1979. Human leukocyte elastase and cathepsin G: structural and functional characteristics. Excerpta Med. **75**: 51–68.
24. **Walstad, D. L., L. F. Guymon, and P. F. Sparling.** 1977. Altered outer membrane protein in different colonial types of *Neisseria gonorrhoeae*. J. Bacteriol. **129**:1623–1627.