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Gonococci with Mutations to Low-Level Penicillin Resistance Exhibit Increased Sensitivity to the Oxygen-Independent Bactericidal Activity of Human Polymorphonuclear Leukocyte Granule Extracts

JUDY A. DALY,[1] TERRENCE J. LEE,[1]† JOHN K. SPITZNAGEL,[2] AND P. FREDERICK SPARLING[1]*

Department of Bacteriology and Immunology and Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514[1]; and Department of Microbiology, Emory University School of Medicine, Atlanta, Georgia 30322[2]

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Gonococci which cause disseminated gonococcal infection are nearly always highly penicillin sensitive, in contrast to many isolates causing uncomplicated gonorrhea. We questioned whether any of the known chromosomal mutations to low-level penicillin resistance might adversely affect virulence. The *penA2* locus is known to result in low-level resistance to penicillins, whereas *mtr-2* results in nonspecific resistance to a variety of antimicrobial agents. We found that the *penA2* and *mtr-2* mutations each markedly increased sensitivity of strain FA19 to oxygen-independent killing by human polymorphonuclear leukocyte mixed or isolated azurophilic granule extracts. The *penA2* and *mtr-2* mutations had no effect on sensitivity to serum antibody and complement. Isogenic opaque or transparent variants of several strains of *Neisseria gonorrhoeae* were equally resistant to human polymorphonuclear leukocyte mixed granule extract bactericidal systems. There were also no differences in susceptibility of piliated type 1 and nonpiliated type 4 variants to human polymorphonuclear leukocyte mixed granule extracts. Since the *penA2* and *mtr-2* loci are known to increase the degree of cross-linking of cell wall peptidoglycan, the structure of peptidoglycan apparently affects sensitivity to killing by one or more polymorphonuclear leukocyte azurophilic granule extract bactericidal systems. These observations might explain why gonococci with mutations similar to *penA2* and *mtr-2* are almost never isolated from patients with disseminated gonococcal infection.

Pathogenicity of *Neisseria gonorrhoeae* is known to be related to cell surface structure. Virulence is closely correlated with presence of type 1 pili (11), probably because pili increase attachment to mucosal cells (31) and decrease ingestion and killing by neutrophils (4). Heat-modifiable outer membrane proteins (protein II) associated with colony opacity (13, 30, 32) also apparently affect mucosal attachment (31) and the ability to cause disseminated gonococcal infection (DGI) (10). Most gonococci isolated from patients with DGI have the same serotype of outer membrane protein I (1). Other bacterial phenotypes strongly associated with gonococcal invasiveness include resistance to pooled normal human serum (26) and penicillin sensitivity (7). Isolates from patients with DGI are much more sensitive to penicillin and certain other antibiotics than are isolates from patients with uncomplicated gonococcal infection (7). Genetic and epidemiological studies have demonstrated that penicillin sensitivity is not directly related to any of the other properties associated with invasiveness (3, 7). A pathogenic basis for the correlation between penicillin sensitivity and virulence has not been proposed.

Low-level resistance of gonococci to penicillin G is known to result from additive effects of several chromosomal mutations. Some of these mutations affect the structure of one or another of the outer membrane constituents (9), the peptidoglycan (9), or the cytoplasmic membrane (5). Certain mutations which affect cell envelope structure and increase penicillin G resistance in *Salmonella typhimurium* (20) are known to decrease virulence for mice (20). These mutations increase susceptibility to phagocytosis (29), killing by serum antibody and complement (19), and killing by isolated polymorphonuclear leukocyte (PMN) granule extracts (23) and decrease the ability to scavenge available iron (12). Decreased virulence could be due to one or more of these effects. Cell surface mutations are also

† Present address: Asheville Infectious Disease Associates, Asheville, NC 28801.

known to affect the virulence of *Escherichia coli* (18) and other bacteria. These observations stimulated us to study some of the possible effects of gonococcal mutations to penicillin G resistance on pathogenicity.

In this paper, we present evidence that gonococcal mutations to low-level penicillin G resistance markedly increased sensitivity to killing in vitro by human PMN azurophilic granule extracts.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains are listed in Table 1. Most have been extensively characterized in this laboratory previously (9, 25, 27). The parent strain (FA19) used as the recipient in the construction of isogenic antibiotic-resistant transformants is resistant to normal human serum and is highly antibiotic sensitive. It is known to be virulent because it has caused DGI in a laboratory worker after pharyngeal inoculation by a mouth-pipetting accident (unpublished data). Strains were stored at −70°C in Trypticase soy broth (BBL Microbiology Systems) containing 20% glycerol and subcultured from the frozen state. Strains were type 1 (piliated) transparent colonies unless otherwise indicated.

Media and growth. Routine subculture was on GCB agar (GCBA; Difco Laboratories) which contained 1% (vol/vol) Kellogg defined supplements 1 and 2, as previously described (27). Broth cultures were in GCB broth (GCBB) containing 1% (vol/vol) defined supplements 1 and 2; GCBB was identical to GCBA except for the omission of starch and agar. In some assays, minimal medium Davis (Difco) was used for serial dilutions. All agar grown cultures were incubated at 37°C in a humidified stationary chamber containing 3 to 5% CO_2.

Isolation of normal human PMN granules. Granules were separated essentially as described by Spitznagel et al. (28). Leukocytes were obtained from healthy donors between the ages of 18 and 30 with normal hematocrit and normal total and differential leukocyte counts. These studies were approved by the Committee on the Protection of the Rights of Human Subjects at the University of North Carolina. Most erythrocytes were removed by sedimentation through Plasmagel or by dilution with two volumes of 3% dextran (250,000 daltons). PMNs were further purified by Ficoll-Hypaque sedimentation at 400 × *g* for 35 min at 20°C by the method of Böyum (2). Final cell suspensions contained ≥90% PMNs, with ≤10% contaminating lymphocytes, monocytes, and eosinophils as ascertained by Wright-stained pulled blood smears and cytocentrifuge slides. One unit of blood yielded approximately 1.0×10^9 PMNs. PMNs were suspended in 0.34 M sucrose and ruptured by homogenization. Whole cells, nuclei, and aggregated cell debris were removed by centrifuging at 126 × *g* for 15 min.

Mixed granule isolation. The 126 × *g* supernatant was centrifuged at 20,000 × *g* for 20 min to yield a pellet containing a mixed population of granules (two classes of azurophil granules and one class of specific granules). Subsequently the mixed granules were extracted twice overnight at 4°C in 0.2 M sodium acetate buffer (pH 4.0) containing 0.01 M $CaCl_2$ by gently stirring. At 24 h before granule extracts were to be used, they were dialyzed free of acetate against phosphate-buffered normal saline (7.4 g of NaCl, 0.285 g of KCl, 0.29 mg of Na_2HPO_4, and 0.083 g of KH_2PO_4 per liter of deionized water, pH 7.0) in an Amicon MMC concentrator/dialyzer over a UM-2 or YM-2 Amicon membrane (molecular weight cutoff, 1,000) or in dialysis tubing (molecular weight cutoff: 3,500). Granules from 1.0×10^9 PMNs yielded about 10 mg of extract protein.

Isolation of specific and azurophilic granules. Specific and azurophilic granules were prepared from 5 U (2,500 ml) of blood. Mixed granules were prepared from 500-ml volumes by plasmagel sedimentation, centrifugation through Ficoll-Hypaque, hypotonic lysis of contaminating erythrocytes, and homogenization as described above. The homogenates (in 0.34 M sucrose) were pooled and adjusted to 25% (wt/vol) sucrose by dropwise addition of 60% (wt/vol) sucrose and separated over linear (30 to 53%, [wt/vol]) sucrose gradients by isopycnic centrifugation to yield one population of specific granules and two populations of azurophil granules, as described previously (24). Separated granules were located by measurement of optical density at 450 nm, and their identity was verified by demonstrating the association of specific granules with

TABLE 1. Strains of *N. gonorrhoeae* used[a]

Strain	Genotype	Phenotype	Cell envelope chemistry
FA19	Wild type	Antibiotic sensitive	
FA102	*penA2*	Resistant to penicillins	Increased peptidoglycan cross-linking
FA171	*mtr-2*	Resistant to antibiotics, dyes, detergents	Increased peptidoglycan cross-linking, increased amounts of a 52,000-dalton outer membrane protein
FA136	*penA2*, *mtr-2*	Resistant to penicillins, tetracycline, other antibiotics, dyes, detergents	Increased peptidoglycan cross-linking, increased amounts of a 52,000-dalton outer membrane protein
FA140	*penA2*, *mtr-2*, *penB2*, *nmp-1*	Resistant to penicillins, tetracycline, other antibiotics, dyes, detergents	Increased peptidoglycan cross-linking, increased amounts of a 52,000-dalton outer membrane protein, increased molecular mass of principal outer membrane protein

[a] The origins, phenotypes, and cell envelope chemistry of these strains have been previously described (3, 9).

lactoferrin and azurophil granules with myeloperoxidase. Granules were pooled, purified, extracted, and concentrated as described above.

Protein determinations. Total protein determinations were made by the method of Lowry et al. (15), with egg white lysozyme (500 μg/ml) as a standard.

Single radial immunodiffusion. Lysozyme and myeloperoxidase were quantified antigenically by the method of Mancini et al. (16). Monospecific rabbit anti-human myeloperoxidase was prepared with human leukemic myeloperoxidase which had been purified on a Bio-Rex ion-exchange column (21). Monospecific rabbit antiserum to human milk lactoferrin was prepared as previously described (14). Antisera were incorporated into a 1.0% agarose solution in phosphate buffer (pH 8.0) containing 1 M NaCl. This solution was poured into a 1-mm-thick mold made with Kodak projection slide cover glass and allowed to solidify. The test sample was mixed in 0.005 ml of 1.03% cetyltrimethyl ammonium bromide, placed in punched wells, and incubated for 18 to 72 h. Subsequently, the plates were washed with saline, dried, and stained with Coomassie brilliant blue. A standard dilution curve of antigen was run with each assay, and the concentrations of unknown samples were determined from this curve.

Lysozyme. Lysozyme was measured enzymatically by the method of Osserman and Lawlor (22).

Bactericidal assay. Granule extract bactericidal activity was assayed in GCBB by a modification of the assay of Rest et al. (23). Type 1 transparent colonies cultured on GCBA for 16 to 20 h were inoculated into GCBB to an initial density of 20 Klett units (Klett-Summerson colorimeter, no. 540 filter), and were incubated at 37°C in 5% CO_2 (or with 0.005 M $NaHCO_3$) until they reached mid-log phase (80 to 100 Klett units) 3 to 4 h later. In some assays, cells were collected by centrifugation at 4°C, washed once with ice-cold minimal medium Davis, and suspended in GCBB. Cells were then diluted in GCBB to give 10^3 to 10^4 colony-forming units per ml. The bactericidal reaction mixture contained 50 to 100 μl of cells, 20 to 100 μl of granule extract, and sufficient GCBB growth medium to make a final volume of 200 μl. The pH was 7.0 unless otherwise indicated. Controls consisted of cells with GCBB only or cells, GCBB, and phosphate-buffered saline (pH 7.0). Additional controls for individual assays are described below. Assays were performed in wells of plastic microtiter plates (Cooke Laboratories). Incubations were for 60 min at 37°C in a New Brunswick Gyrotory shaker in an atmosphere containing 3 to 5% added CO_2 or with 0.005 M $NaHCO_3$ added to the reaction mixture. After 48 h, 100 μl of the reaction mixture was spread on GCBA plates and counted. Results are expressed as the percentage of viable colony-forming units remaining at 60 min compared with controls.

Statistical methods. A Wilcoxon Mann Whitney *U* statistic was used to test the significance of single point relationships. The significance of the differences of means of the effect of granule extracts on various transformants was determined by Student's *t* test.

RESULTS

Bactericidal activity of mixed granule extracts. Various amounts of granule extract were incubated for 1 h at 37°C with the antibiotic-sensitive parent strain FA19 and its isogenic penicillin G-resistant transformants FA102, FA171, FA136, and FA140. Each of these transformants was markedly more sensitive to killing by mixed human PMN granule extract proteins than was strain FA19 ($P < 0.0005$) (Fig. 1). Microscopic examination of wet mounts did not show evidence of noticeable differences in mixed granule-induced clumping of the transformants as compared to FA19. The four transformants were not statistically different from each other in this assay. As little as 2 μg of granule extract per ml resulted in an approximate 50% decrease in viability of each of the penicillin G-resistant transformants, whereas the penicillin G-sensitive parent strain FA19 was not affected until a concentration of at least 52 μg/ml was attained. Thus, the concentration of granule extract needed to initiate killing of penicillin G-resistant transformants was only 3% of that required for the same effect on the penicillin G-sensitive parent strain FA19. Sixty-six percent of the colony-forming units of strain FA19 survived exposure to 206 μg of granule extract protein per ml for 60 min, whereas at most only 7% of the colony-forming units of strains FA102 (*penA2*), FA171 (*mtr-2*), FA136 (*penA2*, *mtr-2*), and FA140 (*penA2*, *mtr-2*, *penB2*, *nmp-1*) survived under the same conditions.

Strains FA102 (*penA2*) and FA171 (*mtr-2*) were equally sensitive to mixed PMN granule extracts. The *penA2* and *mtr-2* mutations each result in low-level penicillin G resistance, but are otherwise genetically, phenotypically, and biochemically distinct (9, 27). Combination of

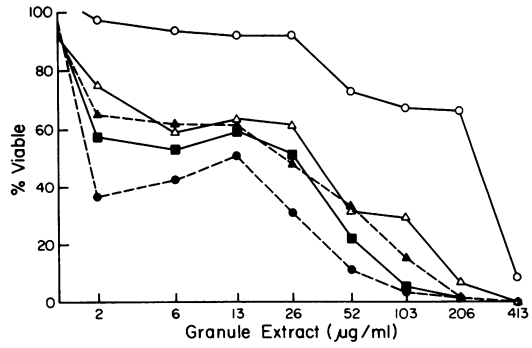

FIG. 1. Effect of mixed granule extracts on strain FA19 and its penicillin G-resistant transformants. Values represent the mean of 8 to 12 determinations. Symbols: strain FA19 (wild type), ○; strain FA102 (*penA2*), ●; strain FA171 (*mtr*), △; strain FA136 (*penA2*, *mtr-2*), ▲; strain FA140 (*penA2*, *mtr-2*, *penB2*, *nmp-1*), ■. All penicillin G-resistant transformants were significantly more susceptible to mixed granule extracts than strain FA19 ($P < 0.0005$).

the *mtr-2* and *penA2* loci (strain FA136) did not increase sensitivity to mixed PMN granule extracts (Fig. 1). Likewise, the addition of a third locus (*penB2*) for low-level penicillin G resistance and a closely linked locus (*nmp-1*) which affects the structure of outer membrane protein I (3) (strain FA140) caused no additional effects on sensitivity to PMN granule extracts (Fig. 1).

It was possible that the apparent effects of either *penA2* or *mtr-2* on sensitivity to mixed PMN granule extracts might have resulted from introduction of a linked marker. Accordingly, we constructed another independent *penA2* transformant of strain FA19, using transforming DNA from strain FA48, by previously established methods (27). Transformants exhibiting four- to eight fold-increased Pen resistance, but no effect on sensitivities to other antibiotics, were considered to have received the *penA2* mutation. One such transformant was tested against increasing doses of PMN mixed granule extracts; it displayed sensitivities not significantly different from strain FA102 (*penA2*). This result confirmed that the locus conferring susceptibility to human PMN granules was identical with or closely linked to the *penA2* locus.

The effect of other cell surface variables which might have influenced the results was also examined. The *penA2* and *mtr-2* loci had no effect on the resistance of strain FA19 to killing by serum antibody and complement. Neither variations in piliation nor variations in colony opacity associated outer membrane heat-modifiable protein II composition (30, 32) had any effect on sensitivity of strain FA19 to killing by mixed PMN granule extracts (data not shown). Several mutants of strain FA19 which are altered in the structure of their lipopolysaccharide (kindly supplied by L. Guymon) also were identical or very similar to strain FA19 in their resistance to mixed PMN granule extracts.

Effect of pH on the bactericidal activity of mixed granule extracts. The effectiveness of PMN bactericidal systems varies with pH (17). Therefore, strain FA19 and its *penA2* transformant FA102 were incubated with mixed PMN granule extracts under standard assay conditions, except that the reaction mixture was buffered to pH 5, 6, 7, or 8 with 0.05 M potassium phosphate (Fig. 2). Controls were performed at the respective pHs; viability of controls was not affected during the 60-min incubation of these experiments. Both strains FA19 and FA102 were more susceptible to mixed PMN granule extracts at pH 5; otherwise pH had no effect on susceptibility to granule extracts.

Bactericidal activity of boiled mixed granule extracts. To distinguish between enzymatic and nonenzymatic bactericidal activity, mixed granule extracts were boiled for 30 min. This resulted in nearly total loss of the bactericidal effect of the PMN granule extracts on each of the penicillin G-resistant transformants (data not shown).

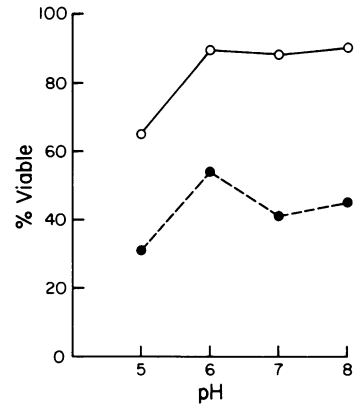

FIG. 2. Effect of pH on the bactericidal activity of mixed granule extracts (62 μg/ml) on strain FA19 and its penicillin G-resistant transformant. Values represent the mean of 8 to 12 determinations. Symbols: strain FA19 (wild type), ○; strain FA102 (*penA2*), ●. Mixed granule extracts were significantly more bactericidal at each pH point for strain FA102 as compared with strain FA19 ($P < 0.0001$).

Granule separation. A typical separation of the different PMN granules by sucrose density gradient centrifugation is shown in Fig. 3. Subcellular granules were resolved into three or four populations. The distribution of specific and azurophil granules was essentially as described by Rest and Spitznagel (24). Specific granules (δ23° = 1.187 g/ml) were located in peak II, which contained all of the PMN lactoferrin and approximately one-half of the lysozyme, but no myeloperoxidase (Table 2). Peak IIIs contained the slowly migrating (light) azurophil granules (δ23° = 1.206 g/ml); these granules contained no lactoferrin, about one-quarter of the cells' lysozyme, and about 40% of the cells' myeloperoxidase (Table 2). Peak IIIf contained the fast-migrating (heavy) azurophil granules (δ23° = 1.222 g/ml); they contained more lysozyme and myeloperoxidase than the slowly sedimenting azurophil granules.

Bactericidal activity of azurophil and specific granule extracts. Various amounts of protein extracted from specific, light azurophil, and heavy azurophil granules were incubated for 1 h at 37°C with strain FA19 (wild type) and its antibiotic-resistant transformant strains FA102 (*penA2*), FA171 (*mtr-2*), FA136 (*penA2*, *mtr-2*), and FA140 (*penA2*, *mtr-2*, *penB2*, *nmp-1*) as described above. Specific granule extracts were not bactericidal for strain FA19 (wild type) or transformants with *penA2* and *mtr-2* mutations until concentrations of ≥180 μg of protein per

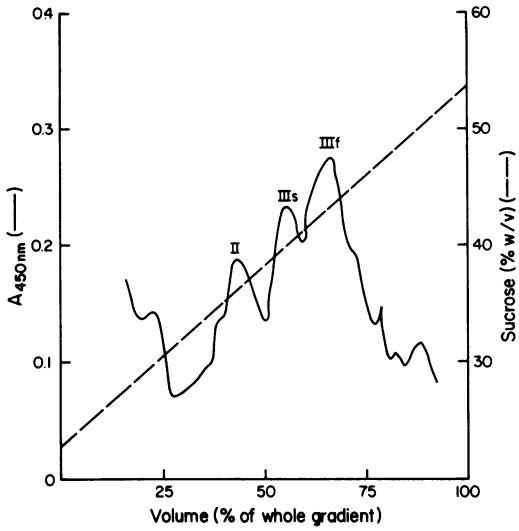

FIG. 3. Linear sucrose (30 to 53% [wt/vol]) gradient of 5-Unit extraction. Absorbance (—) and sucrose (---) profiles of gradient. Fifty fractions of 10 ml were eluted. Key to peaks: II, specific granules; IIIs, light azurophil granules; IIIf, heavy azurophil granules.

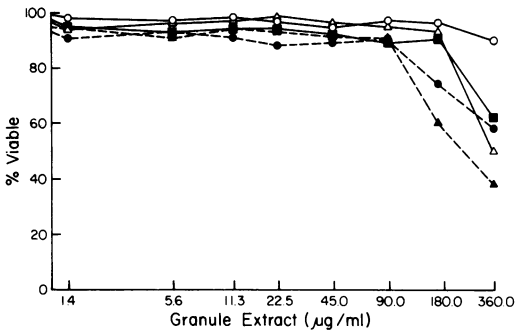

FIG. 4. Effect of peak II specific granule extract on penicillin G-resistant transformants of strain FA19. Values represent the mean of four to eight determinations. Symbols: strain FA19 (wild type), ○; strain FA102 (*penA2*), ●; strain FA171 (*mtr*), △; strain FA136 (*penA2*, *mtr-2*), ▲; strain FA140 (*penA2*, *mtr-2*, *penB2*, *nmp-1*), ■. There were no significant differences ($P > 0.078$) between strain FA19 and its penicillin G-resistant transformants.

ml were reached (Fig. 4). Markedly increased activity was seen with either IIIs or IIIf azurophilic fractions against each of the penicillin G-resistant transformants, as compared with their penicillin G-sensitive parent (Fig. 5). Strain FA19 was not susceptible to fraction IIIs until a concentration of 296.7 μg of protein from IIIs per ml was achieved. However, significant bactericidal effect against each of the transformants was observed at a concentration of 2.3 μg of protein from IIIs per ml. of fraction IIIs. No significant difference in susceptibility was observed between any of the transformants; either the *penA2* or *mtr-2* locus conferred maximum increased sensitivity to killing by azurophilic granule extract. Antibiotic-resistant transformants were as susceptible to IIIf azurophil fraction granule extract as IIIs extracts. Fraction IIIf protein at 2 μg/ml reduced viability of strain FA102 (*penA2*) and strain FA171 (*mtr-2*) significantly, whereas 95% of strain FA19 colony-forming units were still viable when incubated with 130 μg of IIIf protein per ml for 60 min.

Effect of pH on bactericidal activity of specific and azurophil granule extracts. Each strain was incubated for 1 h with specific or azurophilic granule extracts in the GCBB incubation medium buffered to pH 5.0 to 8.0 with 0.05 M potassium phosphate buffer. Controls were performed at the same pH. Peak II specific granule extract was slightly more bactericidal for both strain FA19 and the penicillin G-resistant transformants at pH 5, but no difference was observed between strain FA19 and the other strains. There was markedly increased activity at each pH of IIIs and IIIf azurophil fraction granule extracts against strain FA171 (*mtr-2*) (Fig. 6) and the other penicillin G-resistant transformants (data not shown). The preparation of azurophilic granule extract used for these experiments was consistently more active than

TABLE 2. Composition of phosphate-buffered saline-dialyzed granule extracts[a]

Granule extract	Protein		Lysozyme			Myeloperoxidase			Lactoferrin		
	μg	% Total protein	μg	% Total protein	% Granule protein	μg	% Total protein	% Granule protein	μg	% Total protein	% Granule protein
Specific (II)	7,200	20	2,000	5.6	28.8	0	0	0	1,800	5.1	25
Light azurophil (IIIs)	10,400	29	1,400	4	13.5	1,200	3.4	11.5	0	0	0
Heavy azurophil (IIIf)	17,800	50	2,250	6.3	12.6	1,600	4.5	9	0	0	0

[a] Granule extracts were from 5.0×10^9 PMN from 5 U (2,500 ml) of whole blood.

previous preparations in killing sensitive (but not resistant) strains, for unknown reasons.

Bactericidal activity of boiled azurophil and specific granule extracts. Boiling any of the granule extracts in a water bath for 30 min destroyed all of its anti-gonococcal bactericidal activity.

DISCUSSION

This investigation was prompted by three types of evidence: (i) epidemiological studies have linked penicillin G sensitivity to ability of gonococci to cause bacteremia (7); (ii) chromosomal mutations to penicillin G resistance frequently alter gonococcal cell envelope structure (5, 8, 9); and (iii) in other bacterial species, mutations which alter antibiotic sensitivity and cell envelope structure sometimes diminish ability to resist host defenses (12, 19, 23). It therefore seemed possible that chromosomal penicillin G resistance might interfere in some manner with the ability of gonococci to evade host defenses or reduce their ability to invade vascular tissue.

We found that two genetically distinct chromosomal mutations to low-level antibiotic resistance (*penA2* and *mtr-2*) increased sensitivity of gonococci to nonoxidative killing in vitro by human PMN azurophilic granule extracts. The effects of the *penA2* and *mtr-2* mutations on killing by azurophilic granule extracts were essentially identical and were not additive. The effects observed were probably due to these mutations, rather than some other covariable alteration in gonococcal cell envelope structure, since variations in piliation, colony opacity, and lipopolysaccharide structure had no effect on sensitivity to azurophilic granule extracts. Moreover, several other penicillin G-sensitive DGI isolates were similarly tested, and each was as resistant to PMN granule extract killing in vitro as strain FA19 (data not shown).

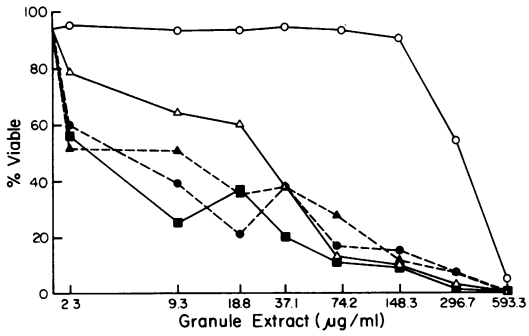

FIG. 5. Effect of IIIs azurophil fraction granule extract on penicillin G-resistant transformants of strain FA19. Values represent the mean of four to eight determinations. Symbols: strain FA19 (wild type), ○; strain FA102 (*penA2*), ●; strain FA171 (*mtr*), △; strain FA136 (*penA2*, *mtr-2*), ▲; strain FA140 (*penA2*, *mtr-2*, *penB2*, *nmp-1*), ■. The IIIs azurophil granule fraction was significantly more bactericidal for penicillin G-resistant transformants than strain FA19 ($P < 0.008$).

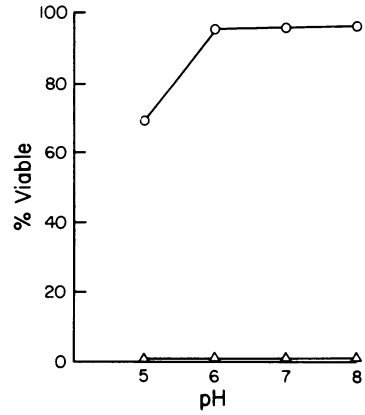

FIG. 6. Effect of pH on the bactericidal activity of IIIs azurophil fraction granule extract (65 μg/ml) on strain FA19 and its antibiotic-resistant transformant. Values represent the mean of four to eight determinations. Symbols: strain FA19 (wild type), ○; strain FA171 (*mtr*), △.

Although increased sensitivity to killing by PMN azurophilic granules is a plausible basis for decreased ability of penicillin G-resistant gonococci to cause DGI, the present data do not prove this point. Mutations similar to *penA2* or *mtr-2* could also alter gonococcal pathogenicity in other respects not examined in these experiments. However, we did exclude the most obvious alternative: *penA2* or *mtr-2* did not increase sensitivity to the bactericidal effect of normal human serum. These mutations also did not increase sensitivity to H_2O_2 in concentrations equivalent to those generated by stimulated human neutrophils (M. S. Cohen, personal communication).

If mutations similar to *penA2* or *mtr-2* diminish the likelihood of developing DGI because they increase gonococcal sensitivity to killing by PMN granules, they might be expected to increase sensitivity to phagocytosis and killing by intact PMNs. We have investigated the rate and extent of phagocytic killing of strain FA19 and strain FA102 by intact human PMNs, using methods similar to those used by several other investigators (4). The results were too variable to draw meaningful conclusions (data not shown). The reasons for our difficulty in obtaining reproducible killing of gonococci by intact PMNs were unclear, but the difficulty may have been due to the large number of variables involved in attachment to and phagocytosis by whole PMNs.

If mutations similar to *penA2* or *mtr-2* decrease virulence by increasing susceptibility to killing by PMNs, it is somewhat surprising that gonococcal isolates associated with salpingitis are apparently more likely to be penicillin G resistant than are uncomplicated disease isolates (6). Salpingitis is also certainly an invasive form of gonococcal infection, and neutrophils are probably important in the host response to gonococcal salpingitis. However, there may be many other factors which determine whether gonococci can cause salpingitis.

The explanation for the *penA2*- or *mtr-2*-associated increase in susceptibility to killing by PMN azurophilic granules is unclear. The *penA2* mutation results in increased resistance to penicillins but not other antimicrobial agents (27), probably by alteration of a cytoplasmic membrane penicillin-binding protein (5). This effect is accompanied by a small increase in cross-linking of the cell wall peptidoglycan (9). The *mtr-2* mutation increases resistance to many diverse antimicrobial agents (27), probably by decreasing cell envelope permeability (8). The *mtr-2* mutation increases the quantity of a 52,000-dalton outer membrane protein and increases cross-linking of the peptidoglycan to the same extent as the *penA2* mutation (9).

Since the only known common alteration due to the *penA2* and *mtr-2* mutations is increased peptidoglycan cross-linking, we tentatively conclude that increased cross-linking may result in increased sensitivity to killing by PMN azurophilic granules. Explanations for the mechanisms involved are highly speculative at present and will depend in part on elucidation of the factor(s) in azurophilic granules involved in killing of gonococci carrying these mutations. Such studies are presently in progress.

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