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Increased Sensitivity of Gonococcal *pilA* Mutants to Bactericidal Activity of Normal Human Serum

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PilA is a pleiotropic transcriptional regulator in *Neisseria gonorrhoeae*, encoded by an essential gene, *pilA*. It regulates pilin gene expression and stress response and it is implicated in gonococcal adaptation to external signals. All these phenomena may participate in gonococcal virulence. In this report, I tested the role of PilA in another aspect of gonococcal virulence, resistance to the bactericidal effect of normal human serum. Gonococcal mutants with impaired PilA function were more susceptible to the bactericidal effect of normal human serum than the isogenic wild-type strain. However, the major outer membrane protein and the lipooligosaccharide, targets for complement-mediated killing by the serum, were unchanged in the mutants. I discuss the role of PilA in modulating gonococcal sensitivity and resistance to normal human serum.

Neisseria gonorrhoeae is encountered only in humans, in whom it provokes local infections at different sites in addition to systemic infections. Gonococci are continually in contact with human extracellular fluids (serum, mucosal secretions, etc.), in which complement activation is supposed to mediate killing of bacteria. However, gonococci have evolved different mechanisms to evade specific and nonspecific human immune responses. In particular, the resistance of gonococci to complement-mediated killing is of crucial importance in gonococcal virulence. In fact, gonococci are resistant to normal human serum (NHS) in vivo; however, this resistance is often lost after subculture (22). Most gonococcal strains causing disseminated gonococcal infection are serum resistant; moreover, variations in clinical manifestations of disseminated gonococcal infection are linked to differences in the degree of resistance of infecting strains to NHS (13).

Gonococcal serum resistance is a complex phenotype involving gonococcal surface structures such as the lipooligosaccharides (LOS) and the major outer membrane protein (Por) (12). Serum resistance can be stable or unstable. The former involves genetic changes affecting the synthesis of surface structures. In this case, only some bacteria within the population survive because only they have appropriate surface components. Unstable serum resistance results, at least in part, from the induction of resistance by a host factor, cytidine 5′-monophospho-*N*-acetylneuraminic acid, via the sialylation of LOS (11). This sialylation is expected to affect the whole bacterial population, but it depends on the structure of LOS (3). Moreover, neither the kinetics nor how sialylation is effected in vivo is well understood.

Gonococci are thought to adapt to different conditions at their infection sites, but little is known about the effect of this adaptation on the serum resistance/sensitivity phenotype. The aim of this report is to analyze this phenomenon. I have previously reported the characterization of the *pilA/pilB* regulatory system, by which PilA and PilB act as a two-component system regulating the expression of the pilin gene (*pilE*) and other, unidentified genes (16). This regulatory system is thought to modulate the adaptation of gonococci to environmental changes (8, 15, 16). *pilA* appears to be an essential gene, as its inactivation by minitransposon insertion is lethal. Only *pilA*$^+$/*pilA*::mTn-*3* heterodiploids, in which the *pilA-pilB* region is duplicated on the chromosome and one copy contains the minitransposon inserted in *pilA* while the other one remains wild type (16, 19), are viable. One of these heterodiploids, *pilA*$^+$/*pilAa*, exhibited reduced piliation. This phenotype results from the negative transdominant effect of the product of the *pilAa* allele on that of the wild-type one (19). Moreover, this heterodiploid is also affected in the regulation of its response to stress conditions as well as in the level of several proteins (17).

This heterodiploid could be a suitable tool with which to analyze the effect of *pilA* on the serum resistance/sensitivity phenotype under different environmental conditions. In this study, I compared the serum sensitivity of a *pilA*$^+$/*pilAa* heterodiploid with that of the corresponding wild-type strain. I show that the heterodiploid appears to be more susceptible to NHS than the wild-type strain, indicating that PilA is implicated, directly or indirectly, in the regulation of this aspect of gonococcal virulence.

MATERIALS AND METHODS

Bacterial strains and media. All gonococcal variants used in this study were derived from *N. gonorrhoeae* MS11 (14) (Table 1). Bacteria were passaged every 18 to 20 h on G medium plates with G supplement (Diagnostics Pasteur). For liquid cultures, GCB liquid medium with supplements was used (7). For *pilA*$^+$/*pilAa* heterodiploids, chloramphenicol (10 μg/ml) was added to the medium to maintain the *pilAa* allele. It is necessary to use chloramphenicol because in the absence of this selection, the heterodiploid reverts at high frequency to a *pilA*$^+$/*pilA*$^+$ homodiploid as a result of loss of the transposon by homogenotization of *pilA* on nonselective medium. This homodiploid has the *pilA-pilB* duplication (without the minitransposon mTn-*3*) (19).

Sera. NHS samples were obtained from three healthy donors with no history of gonococcal infections. The sera were stored in aliquots at −70°C.

Determination of sensitivity to NHS. In each experiment, one colony of the variant to be tested was suspended in 1 ml of GCB liquid medium with supplements and then diluted 1:100 in 2 ml of Hanks' balanced salt solution containing 1 mM $MgCl_2$, 1.26 mM $CaCl_2$, and 1 mg of bovine serum albumin (Sigma) per ml to give a bacterial suspension of about 5,000 to 10,000 CFU/ml. Pooled NHS from three donors (100 μl) was incubated with 900 μl of bacterial

TABLE 1. Gonococcal variants

Variant	Characteristics	Reference
MS11-A	Wild-type variant for *pilA* and *pilB*, contains *pilE1* and *pilE2*; piliated (P^+), no production of opacity proteins (Opa^-)	14
2B	Wild-type variant for *pilA* and *pilB*; Δ*pilE1* and Δ*pilE2*; derived from MS11-A; P^- Opa^-	14
2BO1	Wild-type variant for *pilA* and *pilB*; Δ*pilE1* and Δ*pilE2*; derived from 2B; P^- Opa^+	This work
4.1C	Wild-type variant for *pilA* and *pilB*; Δ*pilE2*; derived from MS11; P^+ Opa^-	14
C521, C531, C551	Three independent *pilA*$^+$/*pilAa* heterodiploids; Δ*pilE1*, Δ*pilE2*; derived from 4.1C; P^- Opa^-	This work
C551G	Revertant of C551, *pilA*$^+$/*pilA*$^+$ homodiploid; Δ*pilE1*, Δ*pilE2*; P^- Opa^-	This work
VO4.5	Wild-type variant for *pilA* and *pilB*; Δ*pilE2*; derived from 4.1C; P^+ Opa^+	This work
VO451	Wild-type variant for *pilA* and *pilB*; Δ*pilE1*, Δ*pilE2*; derived from VO4.5; P^- Opa^+	This work
OP551	Heterodiploid *pilA*$^+$/*pilAa*; Δ*pilE1*, Δ*pilE2*; derived from VO4.5; P^- Opa^+	This work

suspension for the indicated periods of time at 37°C, and 100 μl was plated on G medium plates (supplemented with chloramphenicol [10 μg/ml] for the *pilA*$^+$/*pilAa* heterodiploid). Plates were incubated for 24 h at 37°C in the presence of 5% CO_2. For control assays, the NHS was inactivated at 56°C for 1 h prior to use (ΔNHS), and the bacteria were incubated for the same period of time. Results are expressed as percent killing: [1 − (CFU with NHS/CFU with ΔNHS)] × 100. There was no significant increase or decrease in viable cell counts in the control samples over the incubation period. However, in the study of the kinetics of killing, I used an additional formula to calculate the percent killing: [1 − (CFU with NHS/CFU with NHS at time zero)] × 100. In this case, the number of viable bacteria was compared at each time point with the initial number of viable bacteria at time zero. Both formulas gave comparable results under the experimental conditions used, as the gonococci do not seem to grow significantly in ΔNHS. Unless indicated differently, the first formula was used in this study.

There was no increase or decrease in clumping of the variants tested in the presence of NHS, as determined by phase-contrast microscopy.

Analysis of opacity proteins. Gonococci were harvested and washed and resuspended in 50 mM Tris-HCl (pH 7.5). Bacterial suspensions were adjusted to an optical density at 550 nm (OD_{550}) of 2.5. Bacterial suspension (25 μl) was added to the same volume of 2× sample buffer (0.25 M Tris-HCl [pH 6.8], 40% [vol/vol] glycerol, 6% [wt/vol] sodium dodecyl sulfate [SDS], 8% [vol/vol] 2-mercaptoethanol, 0.005% [wt/vol] bromophenol blue) and heated at 100°C for 5 min. Samples were then analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). The proteins were then visualized by Coomassie brilliant blue staining or transferred to a nitrocellulose filter (Amersham) and hybridized with anti-opacity protein serum.

Analysis of LOS. Bacteria from control plates in the bactericidal assay of NHS were harvested and washed and resuspended in buffer A (20 mM Tris-HCl, 1 mM $MgSO_4$ [pH 7.5]). Bacterial suspension (25 μl) was added to the same volume of 2× sample buffer and heated at 100°C for 5 min. Proteinase K (10 μl of 2.5 mg/ml in 1× sample buffer) was added, and the mixture was incubated for 1 h at 60°C. Samples were then analyzed by SDS-PAGE on a gel containing 15% polyacrylamide and 4 M urea. LOS was visualized by silver staining as described previously (21).

RESULTS

Role of opacity protein and pilin in gonococcal susceptibility to NHS. I first examined the effect of the production of an opacity protein (Opa) or pili on sensitivity to NHS. Opa^+ variants produce an opacity protein, an outer membrane protein, the product of the *opa* gene, the transcription of which is not regulated by PilA (16). The presence or absence of Opa proteins in the variants used in this study was confirmed by their heat-modifiable migration on SDS-PAGE gels (Fig. 1) and by immunoblot analysis with anti-Opa serum (data not shown).

Conflicting results were reported on the effect of piliation on the sensitivity/resistance phenotype (9, 20). In my experience, piliation seems to increase gonococcal serum susceptibility. Two gonococcal isogenic wild-type variants which differ only in the production of pili (piliated, P^+; nonpiliated, P^-) were compared. Variant MS11-A (P^+ Opa^-) was more sensitive to NHS than variant 2B (P^- Opa^-) at a serum dilution of 1:10 and with an incubation period of 30 min (Fig. 2). The same difference was observed between variant VO4.5 (P^+ Opa^+) and variant VO451 (P^- Opa^+) (data not shown).

I next studied the effect of the presence of Opa protein on serum sensitivity. The extent of killing of the 2BO1 (P^- Opa^+) variant was higher than that of 2B (P^- Opa^-). Variant 2BO1 was derived directly from variant 2B (Table 1). Indeed, the presence of Opa protein(s) was previously reported to be associated with increased serum sensitivity in gonococci (5).

I have previously reported that PilA activates *pilE* transcription and that PilA dysfunction in *pilA*$^+$/*pilAa* heterodiploid results in reduced piliation (19). In this study, I examined the role of *pilA* in the serum susceptibility of gonococci independently of the modulation of piliation phenotype by PilA. In order to eliminate any interference of piliation on bacterial sensitivity to NHS, nonreverting nonpiliated (P^- variants deleted on expression site *pilE*) gonococcal variants derived from the MS11 strain were used (Table 1). Moreover, I took care to compare wild-type variants and the corresponding *pilA*$^+$/*pilAa* heterodiploids, which did not differ in production of Opa proteins, i.e., they were either Opa^- or Opa^+ (Table 1). Moreover, the term wild type in this article refers to variants which are wild type for the *pilA* and *pilB* genes.

Increased sensitivity of *pilA*$^+$/*pilAa* heterodiploid to NHS. The killing of different gonococcal wild-type variants was next compared with that of their corresponding *pilA*$^+$/*pilAa* heterodiploids. A serum dilution of 1:10 was used to evaluate the extent of killing of the wild-type variant VO451 (P^- Opa^+), which was compared with its corresponding P^- Opa^+ *pilA*$^+$/*pilAa* heterodiploid (OP551). A serum dilution of 1:10 and an incubation period of 30 min killed twice as many cells of the heterodiploid as of the wild-type variant (Fig. 3). This difference was even more dramatic among Opa^- variants; the virtually serum-resistant wild-type vari-

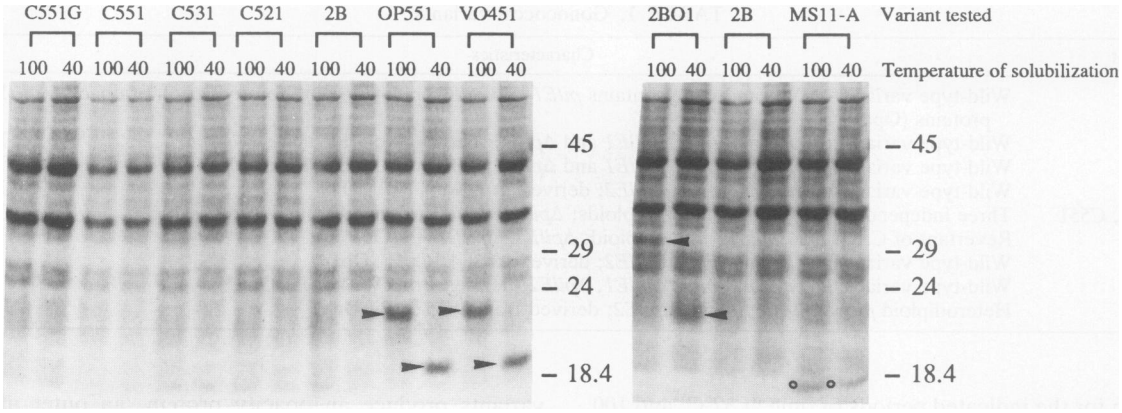

FIG. 1. Electrophoretic mobility of proteins of *N. gonorrhoeae*. Bacteria corresponding to the variants used in this study were harvested and treated as described in Materials and Methods. Samples were then loaded on an SDS-PAGE gel containing 12% polyacrylamide. Proteins were visualized by Coomassie brilliant blue staining. Opas are indicated with arrowheads (variants VO451, OP551, and 2BO1), and pilin is indicated with an open circle (variant MS11-A). Positions of molecular size markers (in kilodaltons) (Sigma) are indicated to the right of each panel.

ant 2B (P^- Opa^-) showed drastically reduced resistance as a result of carriage of a *pilA* mutation (*pilA*$^+$/*pilAa* P^- Opa^- heterodiploid C551). A serum dilution of 1:10 and an incubation period of 30 min killed 1 and 52% of variants 2B and C551, respectively (Fig. 3). Moreover, lower dilution of NHS (1:4) gave 13 and 81% killing for variants 2B and C551, respectively.

Serum diluted to 1:10 was next used to study the kinetics of serum killing. Loss of viability of the wild-type variant VO451 was first observed after 20 min, whereas the heterodiploid OP551 showed five times greater susceptibility

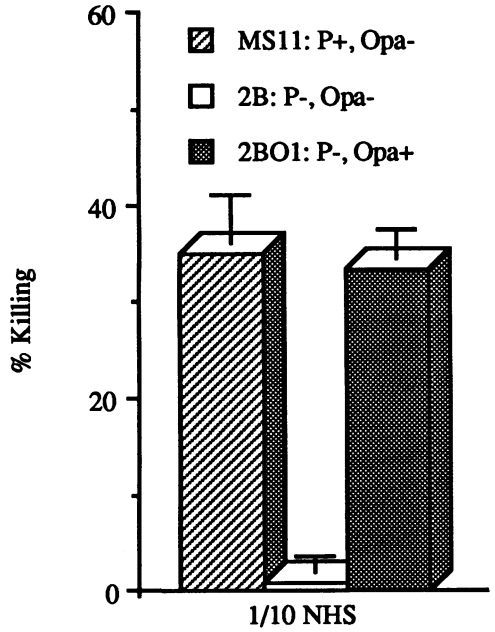

FIG. 2. Killing of *N. gonorrhoeae* by NHS. Bacteria (5,000 to 10,000 CFU/ml) were incubated with a 1:10 dilution of NHS at 37°C for 30 min for the MS11-A (P^+ Opa^-), 2B (P^- Opa^-), and 2BO1 (P^- Opa^+) variants. The extent of killing was determined as described in Materials and Methods. Values represent the mean ± standard deviation (SD) for two independent experiments.

than the wild-type variant. Thereafter, this delay in the kinetics of serum killing of both variants gradually diminished, with almost 100% killing after 60 min in both cases (Fig. 4). The same type of analysis was used to compare the P^- Opa^- wild-type variant 2B with the corresponding P^- Opa^- *pilA*$^+$/*pilAa* heterodiploid C551. It is noteworthy here that prolonged incubation with NHS was needed (Fig. 5). At 30 min, no or very little killing was observed for the 2B variant, while 25 to 50% of the heterodiploid cells were killed. Loss of viability of the wild-type variant was observed at 60 min, and the difference in the extent of killing persisted throughout the experiment (120 min). It is noteworthy that 100% killing was not achieved with the Opa^- variants during this period (Fig. 5). It is interesting that early in the experiment, the slope of the curve for the wild-type variants was slighter than that for the *pilA*$^+$/*pilAa* heterodiploid (Fig. 4, compare OP551 with VO451; Fig. 5, compare C551 with 2B). This indicates a delay in the onset of killing for the wild-type variants.

The presence of chloramphenicol had no effect in serum bactericidal assays with the *pilA*$^+$/*pilAa* heterodiploid as there was no loss of viability in controls in which the bacteria were incubated with chloramphenicol and ΔNHS (data not shown). Moreover, I tested the extent of killing of OP551 and the C551 *pilA*$^+$/*pilAa* heterodiploid in the absence of chloramphenicol by plating the bacteria on G medium without chloramphenicol. Compared with their wild-type variants (VO451 and 2B, respectively), the same difference in the extent of killing was observed as in the presence of chloramphenicol (Fig. 3). This indicates that the loss of viability is the result of complement activation and that it is unlikely that chloramphenicol and serum act additively or synergistically.

These data indicate that both heterodiploids, OP551 and C551, are more susceptible to NHS than their respective corresponding wild-type variants, VO451 and 2B. Moreover, the Opa^- variants are less susceptible than the Opa^+ variants. The most pronounced effect of *pilA*$^+$/*pilAa* is observed in the Opa^+ background, with a significant delay in the onset of killing after exposure to NHS.

Target insertion of *pilAa* allele not responsible for the greater sensitivity of *pilA*$^+$/*pilAa* heterodiploid to NHS. In the *pilA*$^+$/*pilAa* heterodiploids, a *pilA-pilB* duplication contain-

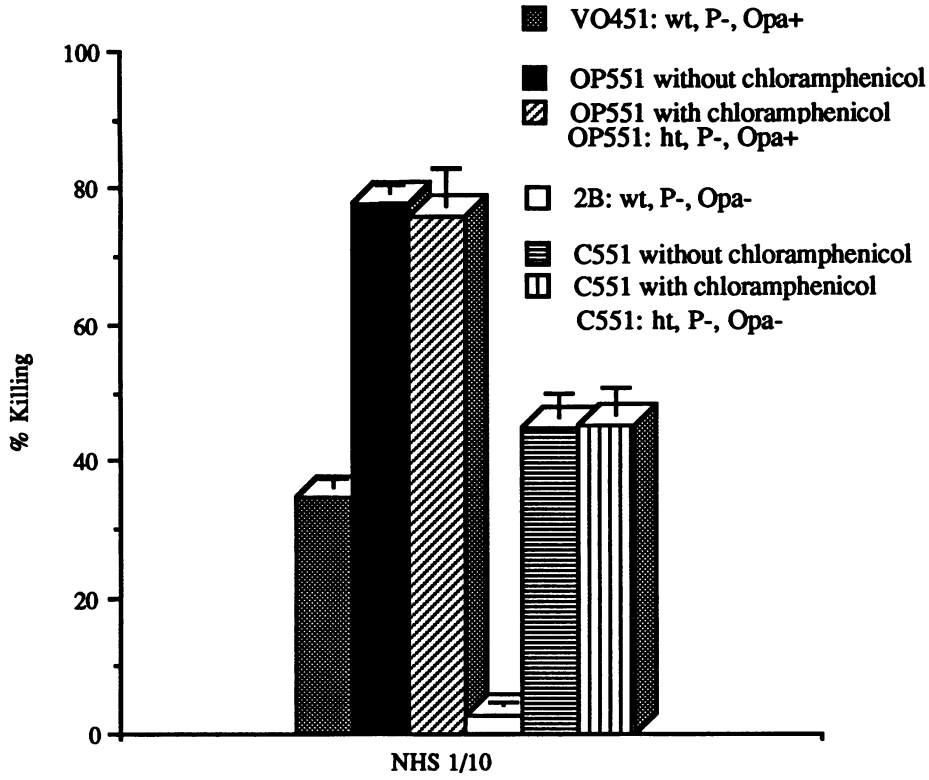

FIG. 3. Killing of P⁻ *N. gonorrhoeae* by NHS. Bacteria (5,000 to 10,000 CFU/ml) were incubated with a 1:10 dilution of NHS at 37°C for 30 min, and the extent of killing was determined as described in Materials and Methods. Nonreverting nonpiliated (P⁻) derivatives of strain MS11 were used. These variants do (Opa⁺) or do not (Opa⁻) produce an opacity protein. wt, wild type; ht, heterodiploid *pilA*⁺/*pilAa*. Values represent the mean ± SD for three independent experiments.

ing the minitransposon had occurred in at least four other chromosomal locations distinct from the wild-type locus, which was conserved on the chromosome (19). This raised the possibility that the observed greater sensitivity of the *pilA*⁺/*pilAa* heterodiploid to the bactericidal effect of NHS could be due to insertional inactivation of a target gene by the *pilA-pilB* duplication rather than to dysfunction of PilA. To rule out this possibility, I tested the sensitivity of three independent heterodiploids (C521, C531, and C551; all are P⁻ Opa⁻) which differ in the location of the *pilA-pilB* duplication (data not shown) (15). Compared with variant 2B (P⁻ Opa⁻), all three heterodiploids were more susceptible to NHS (dilution of 1:10) (Fig. 6).

I next compared the kinetics of killing of the *pilA*⁺/*pilAa* heterodiploid C551 and its *pilA*⁺/*pilA*⁺ homodiploid C551G (P⁻ Opa⁻), which resulted from homogenotization of *pilA* on nonselective medium. This homodiploid still has the *pilA-pilB* duplication (without the minitransposon mTn-*3*) on the chromosome at the same location as the *pilA*⁺/*pilAa* heterodiploid (19). The loss of the transposon reduced NHS susceptibility to the same level as that of the nonpiliated variant 2B, i.e., the loss of the transposon and the restoration of PilA function abolished the hypersensitivity of the heterodiploid C551 to NHS (Fig. 5).

These data, taken together, strongly suggest that the greater sensitivity of the *pilA*⁺/*pilAa* heterodiploid is not related to the location of the *pilA-pilB* duplication but rather to the impaired function of PilA.

LOS profiles of *pilA*⁺/*pilAa* heterodiploid and wild-type strain are identical. Changes in LOS structure and in the Por protein have been shown to be implicated in gonococcal serum resistance; changes in the apparent molecular masses of these two gonococcal surface structures were correlated with modification of sensitivity/resistance phenotype to the bactericidal effect of the NHS (12).

I analyzed LOS in the wild-type variants 2B, 2BO1, and VO451; in the *pilA*⁺/*pilAa* heterodiploids C521, C551, and OP551; and in the corresponding *pilA*⁺/*pilA*⁺ homodiploid C551G. Gonococci were incubated in the presence of ΔNHS and then plated on G medium (supplemented with chloramphenicol for the heterodiploids). LOS were prepared and analyzed by electrophoresis (see Materials and Methods). In all cases tested, the same migration profile was observed, i.e., the same major band was usually observed by this technique (Fig. 7). Moreover, this profile was also observed when LOS were analyzed by immunoblot with NHS (data not shown).

I next analyzed the electrophoretic mobility of the major outer membrane protein Por on an SDS-PAGE gel. No changes were observed between the wild-type and the *pilA*⁺/*pilAa* heterodiploid variants (data not shown). These data indicate that there was no apparent difference in the electrophoretic mobility of the Por protein or of the LOS between the wild-type variant and its corresponding heterodiploid which could be correlated with the increase in serum susceptibility of the *pilA*⁺/*pilAa* heterodiploids.

DISCUSSION

Studies on sensitivity and resistance of gonococci to NHS have focused on the role of LOS and of the major outer membrane protein Por (12). Little is known about the role of

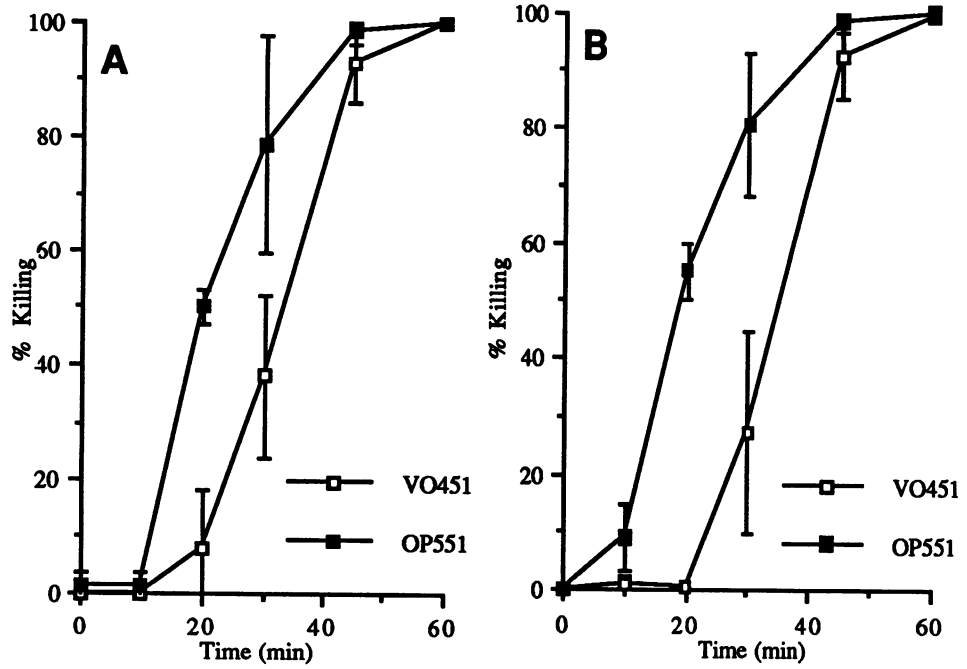

FIG. 4. Kinetics of killing of *N. gonorrhoeae* by NHS. The VO451 variant (Opa$^+$ P$^-$ derivative of strain MS11) and its corresponding *pilA*$^+$/*pilAa* heterodiploid, OP551, were used. Bacteria (5,000 to 10,000 CFU/ml) were incubated with a 1:10 dilution of NHS at 37°C, and samples (100 μl) were taken at the indicated times to determine the extent of killing by plating on G medium (VO451) or G medium supplemented with chloramphenicol (10 μg/ml) (OP551). Values represent the mean ± SD for two independent experiments. (A) The number of viable bacteria was compared with that of controls for each point. (B) The number of viable bacteria was compared at each point with the initial number of viable bacteria at time zero (see the formulas in Materials and Methods).

the other outer membrane proteins (pilins or opacity proteins) or the minor outer membrane proteins. Hitherto, this phenotype has often been analyzed as a static phenomenon, with strains classified as being either sensitive or resistant to NHS because of stable genetic changes affecting surface structures such as LOS and Por protein.

The results with isogenic variants indicate that piliation increases gonococcal susceptibility to NHS. Moreover, Opa$^-$ variants are less susceptible to killing by NHS than Opa$^+$ variants. This confirms previous results (5) that gonococcal strains lacking Opa proteins are more serum resistant than those bearing Opa proteins. The basis of this phenomenon is not clear. The Opa proteins (and pili) may be a target for the activation of complement. Alternatively, the presence of these proteins on the bacterial surface may modify the topology of another target (such as LOS) to permit more efficient bactericidal activity by the complement system. In fact, Opa proteins have been reported to interact with LOS (2). It is interesting that pili and Opa proteins undergo phase variation between P$^-$ and P$^+$ and between Opa$^-$ and Opa$^+$ states. These data indicate a pathophysiological role for the phase variation in gonococci and in particular a role for the Opa$^-$ P$^-$ variants. As pili and Opa proteins are implicated in gonococcal adhesion, reversible Opa$^-$ P$^-$ variants which arise by phase variation are expected to be less adherent and more resistant to the bactericidal effect of complement. These variants are more capable of spread from one anatomical site to another. The reappearance of pili and Opa proteins (Opa$^+$ P$^+$) by phase variation is essential for the full expression of virulence at the new site.

If serum sensitivity is defined as a 70% reduction in CFU after 45 min of exposure to serum at 37°C, then the wild-type Opa$^+$ variant and the corresponding *pilA*$^+$/*pilAa* heterodip-

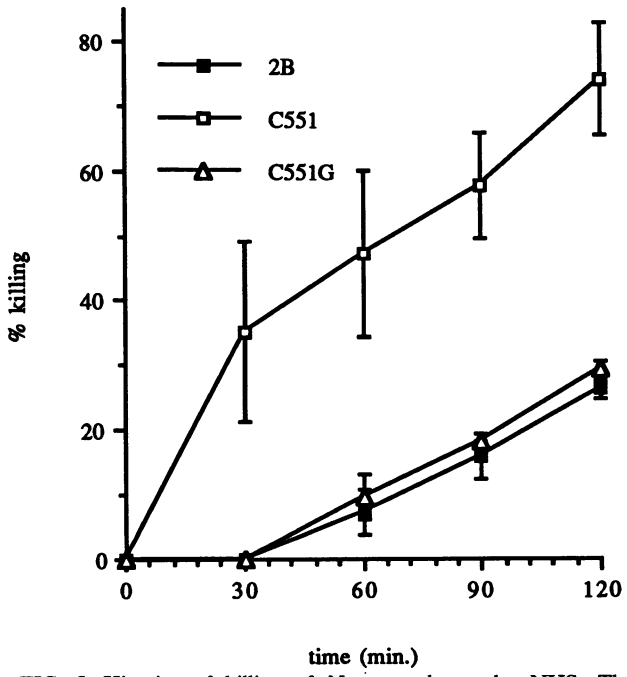

FIG. 5. Kinetics of killing of *N. gonorrhoeae* by NHS. The variants used were 2B; C551, the corresponding *pilA*$^+$/*pilAa* heterodiploid; and C551G, its revertant *pilA*$^+$/*pilA*$^+$ homodiploid (all are P$^-$ Opa$^-$). Bacteria (5,000 to 10,000 CFU/ml) were incubated with a 1:10 dilution of NHS at 37°C, and samples (100 μl) were taken at the indicated times to determine the extent of killing by plating on G medium (2B and C551G) or G medium supplemented with chloramphenicol (10 μg/ml) (C551). Values represent the mean ± SD for two independent experiments.

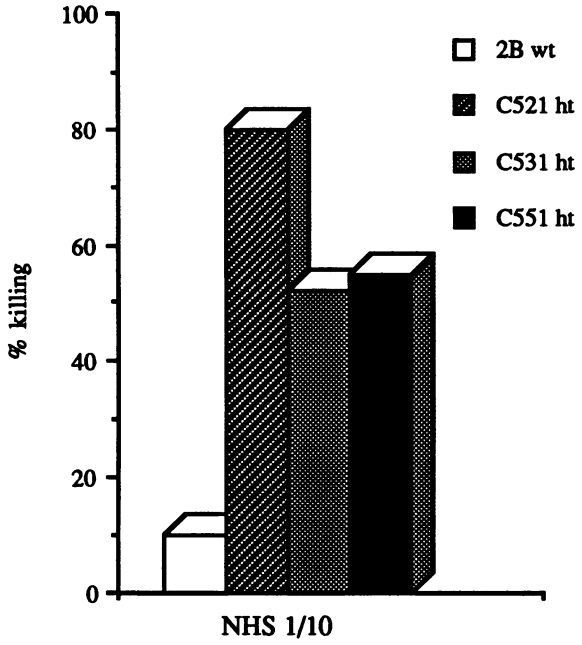

FIG. 6. Extent of killing of *N. gonorrhoeae* by NHS. The 2B variant (P^- Opa^-) and three independent *pilA*$^+$/*pilAa* heterodiploids (C521, C531, and C551; all are P^- Opa^-) were used. Bacteria (5,000 to 10,000 CFU/ml) were incubated with a 1:10 dilution of NHS at 37°C for 45 min. Samples were plated on G medium (2B) or G medium supplemented with chloramphenicol (10 μg/ml) (C521, C531, and C551). Results are from one representative experiment. wt, wild type; ht, heterodiploid.

loid are equally sensitive. However, the results clearly show that the heterodiploid is more rapidly killed by NHS because of an earlier effect of the bactericidal activity of NHS. This difference in the kinetics of killing is important, as variants that are more sensitive to NHS would be expected to be eliminated rapidly, before being able to establish infection at a particular site or to spread to another site. The case of Opa^- variants is quite different, as the wild-type variant is

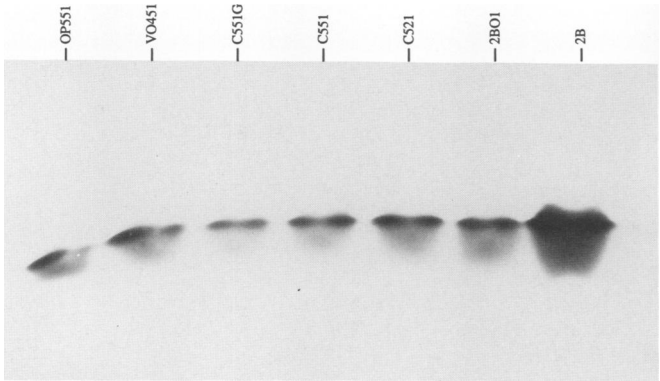

FIG. 7. Electrophoretic mobility of LOS of *N. gonorrhoeae*. Bacteria were harvested from control plates from assays of complement-mediated killing and treated as described in Materials and Methods. Samples were then loaded on an SDS-PAGE gel containing 15% polyacrylamide and 4 M urea. LOS were visualized by silver staining.

resistant, but the *pilA*$^+$/*pilAa* heterodiploid became almost sensitive.

Irrespective of the presence of an Opa protein, *pilA*$^+$/*pilAa* heterodiploids are more sensitive to NHS than the corresponding wild-type variants. One hypothesis for this difference is that the synthesis of one or both major targets (Por and LOS) for complement-mediated bactericidal activity is affected in the heterodiploid. However, neither the LOS nor the Por protein was apparently modified in the heterodiploid. Moreover, I did not observe any difference between the wild type and the heterodiploid variants in the profiles of gonococcal proteins recognized by NHS in an immunoblot analysis (data not shown).

These findings lead me to propose the hypothesis that the *pilA* mutation modifies the level of a minor membrane protein which directly (as a target) or indirectly affects the efficiency of complement activation. In fact, I have previously reported that the level of many proteins is altered in the heterodiploid (17). Moreover, the *pilA*$^+$/*pilAa* heterodiploid is affected in its stress response regulation, as indicated by the overproduction of a GroEL-like protein when the gonococcus is grown on plates (17). Inside the host, *N. gonorrhoeae* is continually exposed to a variety of stress conditions. However, the demonstration of a direct role of the stress response of *N. gonorrhoeae* in the modulation of resistance to the bactericidal effect of the complement system must await further studies.

These results present a dynamic view of the serum sensitivity/resistance phenomenon in *N. gonorrhoeae* by which the kinetics of serum killing is thought to be modulated by the gonococcus. Few studies have been done to examine the influence of the environment on gonococcal sensitivity to complement-mediated killing (6, 10). However, Frangipane and Rest recently reported that anaerobic growth induces high-level serum resistance in *N. gonorrhoeae* (4). Growth conditions can also modulate the sensitivity of *Neisseria meningitidis* to serum bactericidal activity (1). However, the authors did not find any consistent change in the synthesis of outer membrane components that correlated with the modification of serum sensitivity.

The *pilA-pilB* pathway would most likely be involved in the response to environmental factors. The results reported here on the greater sensitivity of the *pilA*$^+$/*pilAa* heterodiploid to NHS should enable analysis of this aspect of bacterial virulence among pathogenic *Neisseria* strains, since the *pilA/pilB* system is conserved in the genus *Neisseria* (18). The data present evidence of coordinate regulation by PilA of piliation and resistance to the bactericidal effects of NHS. Fitness to the infected host (infectivity) and spread inside the same host or between hosts (transmissibility) could be favored by such coordinate regulation of virulence factors.

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