GNA33 of Neisseria meningitidis Is a Lipoprotein Required for Cell Separation, Membrane Architecture, and Virulence

Jeannette Adu-Bobie,1 Pietro Lupetti,2 Brunella Brunelli,1 Dan Granoff,3 Nathalie Norais,1 Germano Ferrari,1 Guido Grandi,1 Rino Rappuoli,1* and Mariagrazia Pizza1

IRIS, Chiron Vaccines,1 and Unit of Electron Microscopy and Cryootechniques, Dipartimento Biologia Evolutiva, University of Siena,2 53100 Siena, Italy, and Children’s Hospital Oakland Research Institute, Oakland, California 946093

Received 8 August 2003/Returned for modification 17 October 2003/Accepted 18 December 2003

GNA33 is a membrane-bound lipoprotein with murein hydrolase activity that is present in all Neisseria species and well conserved in different meningococcal isolates. The protein shows 33% identity to a lytic transglycosylase (MltA) from Escherichia coli and has been shown to be involved in the degradation of both insoluble murein sacculi and unsubstituted glycan strands. To study the function of the gene and its role in pathogenesis and virulence, a knockout mutant of a Neisseria meningitidis serogroup B strain was generated. The mutant exhibited retarded growth in vitro. Transmission electron microscopy revealed that the mutant grows in clusters which are connected by a continuous outer membrane, suggesting a failure in the separation of daughter cells. Moreover, sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of culture supernatant revealed that the mutant releases several proteins in the medium. The five most abundant proteins, identified by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry analysis, belong to the outer membrane protein family. Finally, the mutant showed an attenuated phenotype, since it was not able to cause bacteremia in the infant rat model. We conclude that GNA33 is a highly conserved lipoprotein which plays an important role in peptidoglycan metabolism, cell separation, membrane architecture, and virulence.

The shape and osmotic stability of most bacteria are maintained by the polymeric glycopeptide murein (peptidoglycan), a network of glycan strands interlinked by short peptides. Expansion of the cell wall during bacterial growth and splitting of the septum requires the coordinate action of enzymes which cleave covalent bonds within the murein sacculus and add new peptidoglycan units. For Escherichia coli, it has been proposed that a multienzyme complex (hydrolases and synthases) could be responsible for such a task (9). Thus, murein hydrolases, such as murein lytic transglycosylase A (MltA), are likely to be involved in the enlargement of the murein net and therefore to be essential for growth and division of the bacterial cell wall to allow separation of the daughter cells. Lytic transglycolases are a lysozyme subclass of murein hydrolases which not only cleave the β1-4 glycosidic bond between N-acetylmuramic acid and N-acetylglycosamine but also transfer the glycosyl bond onto the C-6 hydroxyl group of the terminal muramyl moiety, thus forming a 1,6-anhydrodissaccharide tri- and tetrapeptide reaction product demonstrated by incorporation of [3H]palmitate (11). The functional homology of GNA33 with MltA has been confirmed, since the recombinant protein is able to degrade both insoluble murein and unsubstituted glycan strands (11). The formation of 1,6-anhydrosaccharide tri- and tetrapeptide reaction products demonstrated that GNA33 is a lytic transglycosylase (11). In E. coli an MltA deletion mutant as well as mutants with double or triple mutations in the other lytic transglycosylases (MltB and Slt70) showed no obvious phenotype (6, 16, 21). Compared to the case for rod-shaped bacilli such as E. coli, little work has been done to understand the cell division process in gram-negative cocci.

To further define the function of GNA33 in N. meningitidis and its role in growth and cell separation, a knockout mutant was made. Here, we describe the construction and characterization of this mutant and show that GNA33 is essential for cell separation, membrane architecture, and virulence.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Neisseria meningitidis strains MC58, NMB, and BZ232 and their respective gna33 mutants were grown on GC agar base or in GC broth with supplements at 37°C in 5% CO2, unless otherwise stated. For selection of transformants, erythromycin was used at a concentration of 7 μg/ml. E. coli strain DH5 was used for cloning and grown on Luria-Bertani agar base or in Luria-Bertani medium supplemented with ampicillin (100 μg/ml) or erythromycin (200 μg/ml).

Construction of ΔGNA33 mutants of N. meningitidis. Mutants of strains MC58, NMB, and BZ232 (MC58 ΔGNA33, NMB ΔGNA33, and BZ232 ΔGNA33, respectively) in which the gna33 gene was replaced by allelic exchange with an antibiotic cassette were prepared by transforming the parent strain with the plasmid pBSUDGNA33ERM. This plasmid contains upstream and downstream flanking regions for allelic exchange, a truncated gna33 gene, and the ermC gene.
in a humidified atmosphere containing 5% CO\textsubscript{2} to an OD\textsubscript{620} of 0.5. Bacterial growth indicating that the gene was replaced by a 1.2-kb fragment resulting in the MC58, MC58\textunderscore{\textit{mycin}} per ml. PCR with forward primer 5\textsuperscript{'}-GCTCTAGAGATGAGTCGAACAC and reverse primer SD2 
\textsuperscript{SDS} sample buffer (0.06 M Tris-HCl [pH 6.8], 10% [vol/vol] glycerol, 2% [wt/vol] PBS containing 1% (wt/vol) BSA and 1% (wt/vol) Tween 20. Antibody bands were detected by using rabbit anti-mouse immunoglobulin (immunoglobulins G, A, and M)-alkaline phosphatase conjugate polyclonal antibody (Zymed, South San Francisco, Calif.) and Sigma Fast 5-bromo-4-chloro-3-indophenylphosphate-nitroblue tetrazolium substrate (Sigma Aldrich, St. Louis, Mo.).

MALDI-TOF mass spectrometry. Protein bands were excised from the gel, washed with 100 mM ammonium bicarbonate-acetone-tetrahydrofuran (50:50, vol/vol), and dried with a Speed-Vac centrifuge (Savant). The dried spots were digested at 37°C for 2 h by adding 7 to 10 µl of a solution containing 50 mM ammonium bicarbonate, 5 mM dithiothreitol, and 0.012 µl of sequencing-grade trypsin (Promega, Madison, Wis.) per µl. After digestion, 5 µl of 0.1% trifluoroacetic acid was added, and the peptides were desalted and concentrated with Zip-TIP C\textsubscript{18} (Millipore). Samples were eluted onto the mass spectrometer target (Chromaprep 3840 µm; Bruker, Bremen, Germany) with 2 µl of 2.5-dihydroxybenzoic acid (5 g/liter) in 50% triacetin–0.1% trifluoroacetic acid and allowed to air dry at room temperature. Matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) spectra were acquired using a Biflex III MALDI-TOF apparatus (Bruker) equipped with a 337-nm laser and a SCOUT 384 multiprobe. Spectra were acquired in positive mode; acceleration and reflector voltages were set at 19 and 20 kV, respectively. Typically, each spectrum was determined by averaging 100 laser shots. Spectra were externally calibrated by using a combination of four standards (angiotensin II [1,046.54 Da], substance P [1,347.74 Da], bombesin [1,619.82 Da] and ACTH1–39 clip human [2,465.20 Da]) spotted on the probe adjacent to the samples. Protein identification was carried out by both automatic and manual comparisons of experimentally generated monoisotopic peaks of peptides in the mass range of 700 to 3,000 Da with computer-generated fingerprints, using the Mascot program run on proprietary databases.

Growth experiments. All growth experiments were carried out at 37°C with 5% CO\textsubscript{2}. The suspension of organisms was adjusted to an initial OD\textsubscript{600} of 0.05 in 7 ml of GC medium and incubated with continuous end-over-end rotation. The OD was measured every 20 min, and at each time point bacteria were plated out in triplicate to obtain the corresponding CFU per milliliter. Growth studies were performed three times. To determine whether the ΔGNA33 mutant is able to grow in blood, complement-inactivated serum, both the wild-type and mutant strains were grown for 3 h in heat-treated (56°C, 30 min) rabbit serum. Serial dilutions were carried out, and bacteria were plated out to determine CFU.

Transmission electron microscopy. Bacteria were grown in GC medium to an OD\textsubscript{600} of 0.5 and centrifuged, and the pellets were fixed for 1 h in 2.0% glutaraldehyde diluted in PBS. After fixation, the pellets were washed with PBS and then postfixed in 1% osmium tetroxide for 1 h at 4°C. The bacteria were then dehydrated with a graded series of ethanol solutions and embedded in Epon-Araldite. Ultrathin sections were routinely stained with uranyl acetate. The samples were then viewed and photographed in a Philips CM10 transmission electron microscope operated at 80 kV.

Vulnrcin in infant rats. The ability of B232 ΔGNA33 to cause bacteremia was tested in infant rats challenged intraperitoneally (i.p.). The assay was performed as described previously (17). In brief, 5- to 8-day-old pups from litters of outbred Wistar rats (Charles River, Raleigh, N.C.) were randomly redistributed to the nursing mothers. After overnight growth on chocolate agar, several colonies were inoculated into Mueller-Hinton broth (starting OD\textsubscript{600} of 0.05). The broth culture was incubated at 37°C with 5% CO\textsubscript{2} for 24 h in 5 ml of broth and then diluted in PBS with 1% BSA and injected into infant mice infected with 100 μl of the wild-type strain. Blood specimens were obtained 18 h after the bacterial challenge by puncturing the heart with a needle and syringe containing approximately 10 μl of heparin (1,000 U/ml; Fujisawa USA, Deerfield, Ill.) without preservative. Aliquots of 1, 10, and 100 μl of blood were plated onto chocolate agar. The CFU per milliliter was determined after overnight growth at 37°C with 5% CO\textsubscript{2}. To determine whether the mutant is sensitive to heparin, the parent and mutant strains were resuspended in PBS and incubated with or without heparin for 10 min. Bacteria were then plated out to determine the CFU.

RESULTS

GNA33 knockout characterization. A mutation of the gna33 gene was generated in strains MC58, NMB, and B232, and the deletion of the gene was confirmed by PCR with chromosomal DNA. Protein expression was analyzed by immunoblotting in bacterial total lysates or in OMV preparations from wild-type and mutant strains, using anti-GNA33 MAb 25, which recognizes GNA33 and a mimotope present in serotype

\textit{N. meningitidis} LIPOPORTEIN INVOLVED IN CELL SEPARATION

Vol. 72, 2004
P1.2 of PorA (8). The results reported in Fig. 1 show that on the 2996 strain, used as positive control, the MAb recognizes two bands of 41 and 47 kDa. The upper band of 47 kDa corresponds to the GNA33 protein, whereas the lower band of 41 kDa corresponds to the PorA antigen, consistently with the finding that the GNA33 antigen mimics a surface-exposed epitope on loop 4 of PorA in strains of serotype P1.2 (8). The 47-kDa band, corresponding to the GNA33 protein, is present in the OMV preparations and total lysates of MC58 (lanes 2 and 3, respectively) and is absent in the OMV preparation and total lysate of MC58/H9004GNA33 (lanes 4 and 5). In the case of the BZ232 strain, there are two reactive bands in the total cell lysates and OMV preparations (lanes 6 and 7), but only the 41-kDa band is present in the BZ232 ΔGNA33 mutant (lanes 8 and 9), consistent with its P1.2 serotype. The antibodies were not able to recognize GNA33 on the surface of the MC58 strain by fluorescence-activated cell sorter analysis, suggesting that the protein is not surface exposed (data not shown).

The GNA33 mutant exhibits aberrant cell morphology and decreased viability. To evaluate whether GNA33 has any influence on the growth rate or colony morphology of N. meningitidis, the wild-type MC58, NMB, and BZ232 strains and the mutant MC58/H9004GNA33, NMB/H9004GNA33, and BZ232 ΔGNA33 strains were grown on solid or liquid medium. Following overnight incubation of the strains on agar plates, no differences in colony morphology between wild-type and mutant strains could be visualized with the naked eye or with a light microscope (data not shown). To evaluate the influence on growth, the strains were grown for 5 h at 37°C, and samples were collected every 20 min. Each sample was evaluated for OD and for the number of bacterial colonies by plating on GC agar plates. The results of the experiment related to the MC58 and MC58 ΔGNA33 strains are reported in Fig. 2. As shown, the ΔGNA33 mutant exhibits a retardation in growth compared to the parent MC58 strain (Fig. 2a). Furthermore, the number of CFU of MC58 ΔGNA33 per milliliter was much lower than that of the wild-type MC58 strain (Fig. 2b). At an OD_{620} of 0.5, the numbers of viable colonies were $6.7 \times 10^9$ CFU/ml for the MC58 strain and $4.2 \times 10^7$ CFU/ml for the mutant strain, indicating duplication times of approximately 20 min for MC58 and 40 min for MC58 ΔGNA33, resulting in a dramatic reduction in the viable counts. The same results were obtained with the other strains (data not shown).

To verify whether GNA33 plays a role in cell size or cell shape, transmission electron microscopy was used to examine the morphology of the GNA33 mutant. As shown in Fig. 3, the mutant morphology (Fig. 3c and d) is different from the typical diplococcal morphology of the wild-type MC58 (Fig. 3a and b). The mutant grows in clusters (Fig. 3c), and while the inner membrane is separated, the outer membrane remains continuous around the clusters (Fig. 3d), resulting in abnormal cell morphology, with cells of various sizes with an undivided septum. The clusters are represented by cells which are unable to separate. The fact that the mutant grows in clusters is likely to result in misleading outcomes with the colony counts. The bacterial colonies obtained with the mutant strain may be gen-

![FIG. 1. Detection of GNA33 in Western blotting analysis of recombinant GNA33, total lysates, and OMV preparations with anti-GNA33 MAb 25. Lane 1, control strain 2996; lane 2, MC58 OMVs; lane 3, MC58 total lysate; lane 4, MC58 ΔGNA33 OMVs; lane 5, MC58 ΔGNA33 total lysate; lane 6, BZ232 OMVs; lane 7, BZ232 total lysate; lane 8, BZ232 ΔGNA33 OMVs; lane 9, BZ232 ΔGNA33 total lysate; lane 10, recombinant GNA33 from strain 2996. As expected, no reactivity is observed with the mutant strains; however, the antiserum cross-reacts with another band at 47 kDa. This band has been characterized as PorA (8).](http://iai.asm.org/)
erated by clusters of cells and not by individual bacteria. The NMB and BZ232 mutants exhibited the same morphology (data not shown), confirming that the phenotype observed is a result of the deletion of the gna33 gene and not a strain-dependent phenomenon.

The GNA33 mutant releases outer membrane proteins. Since the mutant is impaired in cell separation, we investigated whether the mutation in the gna33 gene may also affect membrane protein assembly. To address this issue, we analyzed the extracellular proteins present in strain MC58 and MC58 ΔGNA33 culture supernatants at an OD620 of 0.5. The culture medium was filtered through a 0.22-μm-size-pore membrane and concentrated by TCA precipitation. Twenty micrograms of protein was separated by SDS-PAGE (Fig. 4). In the culture medium of the mutant strain MC58 ΔGNA33 (Fig. 4, lane 4), several proteins not present in the supernatant of the wild-type strain (Fig. 4, lane 3) were visible on the gel. The five most abundant proteins (Fig. 4, lane 4) were in-gel digested with trypsin and analyzed by MALDI-TOF mass spectrometry for protein identification. The proteins were identified as the outer membrane protein PorA (NMB1429), the major outer membrane PIB (NMB2039), the class 4 outer membrane protein (NMB1053), and a putative adhesin protein (NMB2095). With the exception of NMB2095, the other proteins are also the major proteins identified in the MC58 OMV preparation (Fig. 4, lane 5). The only two faint bands visible in the culture supernatant of the wild-type culture (Fig. 4, lane 3) were both identified as the class 5 outer membrane protein (NMB1053). In conclusion, the deletion in the gna33 gene seems to affect correct topological organization of the N. meningitidis membrane, resulting in the selective release of several outer membrane proteins.

Virulence of the GNA33 knockout mutant. We investigated the ability of the mutant strain to cause bacteremia in infant rats. Since previous experiments had shown that MC58 is unable to cause consistent and reproducible bacteremia in infant rats (data not shown), the BZ232 strain and its GNA33 mutant were used in the study. The virulence experiment was carried out by challenging 5-day-old infant rats i.p with 10⁵ and 10⁶ CFU of BZ232 or BZ232 ΔGNA33. Blood samples were obtained after 18 h and plated onto chocolate agar plates. The results obtained are summarized in Table 1. At both challenge doses of 2.5 × 10⁵ and 2.5 × 10⁶ CFU, the parent BZ232 strain caused bacteremia, while the mutant was unable to cause bacteremia even at the highest challenge dose. As shown in Table 1, all three animals given a challenge dose of approximately 10⁵ CFU of strain BZ232 ΔGNA33 did not exhibit bacteremia after 18 h. In a second experiment, infant rats were challenged with 10⁶ CFU of BZ232 ΔGNA33, and even at this dose the mutant strain failed to cause bacteremia. It is worth noting that the challenge dose of the mutant strain is based on the CFU of the clusters of bacteria, and thus a more bacteria are inoculated than with the wild-type strain.

To verify whether the ΔGNA33 mutant is unable to cause bacteremia because of a susceptibility to killing in presence of heparin or to lyse in the serum, the wild-type and mutant

![FIG. 3. Transmission electron microscopy of MC58 (a and b) and MC58 ΔGNA33 (c and d). While the wild-type parental strain MC58 exhibits a typical diplococci phenotype, the GNA33 mutant shows abnormal cell morphology. Bars, 2 μm (a), 0.4 μm (b and d), and 4 μm (c).](Image)

![FIG. 4. SDS-PAGE of TCA-precipitated culture media and OMV preparation. Lane 1, molecular mass markers; lane 2, 20 μg of MC58 culture medium (OD₆₂₀, 0.5); lane 4, 20 μg of MC58 ΔGNA33 culture medium (OD₆₂₀, 0.5); lane 5, 6 μg of MC58 OMVs.](Image)

**TABLE 1.** *N. meningitidis* strain BZ232 in which GNA33 has been deleted is unable to cause bacteremia and death in infant rats challenged i.p.

<table>
<thead>
<tr>
<th>Exp</th>
<th>Strain</th>
<th>Bacterial dose (CFU)</th>
<th>Results of blood culture 18 h after challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. positive/total</td>
<td>Mean CFU/ml</td>
</tr>
<tr>
<td>1</td>
<td>BZ232 (wild type)</td>
<td>2.5 × 10⁵ 2.5 × 10⁵</td>
<td>4/4 3/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.52 × 10⁶ 1 × 10⁷</td>
</tr>
<tr>
<td></td>
<td>BZ232 ΔGNA33</td>
<td>2 × 10⁵ 2 × 10⁵</td>
<td>0/4 0/3</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>1.4 × 10⁸ 0/4</td>
</tr>
</tbody>
</table>

Downloaded from http://iai.asm.org/ on August 29, 2017 by guest
strains were incubated with or without heparin for 10 min or grown in heat-treated serum for 3 h. For the wild-type strain, $1.43 \times 10^5$ bacteria were obtained in the presence of heparin, compared to $1.36 \times 10^5$ in the presence of heat-inactivated serum. In the case of the mutant strain, $2.6 \times 10^5$ bacteria were obtained in the absence of heparin, compared to $1.3 \times 10^6$ bacteria when incubated with heat-inactivated serum. These results show that the mutant strain is not more susceptible to killing in the presence of heparin than the parent strain. When the mutant was grown in heat-inactivated serum, the numbers of CFU at time zero for the wild-type strain and mutant strain were $2.3 \times 10^7$ and $8.5 \times 10^5$, respectively. After 3 h of incubation, the numbers of CFU for the wild-type strain and mutant strain were $1.7 \times 10^9$ and $4 \times 10^7$, respectively. The results therefore suggest that the mutant strain is able to grow in heat-treated serum. Moreover, the mutant strain exhibits a retardation in growth compared to the parent strain, consistent with data obtained when the strains were grown in GC broth.

**DISCUSSION**

In this study we show that deletion of the *N. meningitidis* gene coding for GNA33, which is homologous to the lytic transglycosylase MltA of *E. coli*, affects cell separation (preventing the formation of daughter cells) cell membrane assembly (causing a release of outer membrane proteins), and virulence. Instead of the characteristic diplococci, the GNA33 mutant shows abnormal cell morphology, which includes an undivided septum, with cells of various sizes growing in clusters of up to 17 cells. The bacterial cells in these clusters have a double septum and a continuous outer membrane.

In *E. coli*, a model for the coordinated metabolism of the murein layer has been suggested (9). The basis of the model is a multienzyme complex consisting of bifunctional murein transglycosylases-transpeptidases (known as penicillin-binding proteins), lytic transglycosylases (Sil70, MltA, and MltB), and MipA (9). It has been suggested that there might be two such complexes, one for the synthesis of murein and another for its degradation. Formation of a complex could be a way for the secure and effective control of potentially autolytic murein hydrolases and to ensure that the growth of the sacculus occurs to maintain the specific shape of the bacterium. Genome computer analysis has shown that *N. meningitidis* has homologs of a majority of the complex members. In addition, by using affinity chromatography, interaction between GNA33 and penicillin-binding protein 2 from *N. meningitidis* serogroup B has been shown (11), further suggesting that GNA33 may be part of a multienzyme complex, as is the case for MltA in *E. coli*. Deletion of three lytic transglycosylases in *E. coli* did not influence growth and division of the mutant *E. coli* strains. In this study we report that deletion of a protein of *N. meningitidis* with homology to the *E. coli* lytic transglycosylase MltA and with a similar in vitro enzymatic activity (11) causes a dramatic effect on growth and cell division. One of the reasons for different phenotypes induced by the deletion of MltA in *E. coli* and GNA33 in *N. meningitidis* may be the different cell shape of the bacterium: *E. coli* cells are rod shaped, while neisseriae are diplococcal. Enzymes shaping the form of the cells may have slightly different roles or be components of different complexes. This observation may represent a first step in the understanding of the mechanisms which regulate cell shapes. The cell division process in gram-negative cocci is essentially uncharacterized. This study suggests that we may learn more by studying the cell shape in cocci. The MinD mutant of *Neisseria gonorrhoeae* (*N. gonorrhoeae* MinD is homologous to the MinD protein of *E. coli* [3, 4]) exhibits a phenotype similar to that of our GNA33 mutant (7). Abnormal cell morphology has been also observed in mutants with mutations in murein hydrolase genes, such as *tep* in *N. gonorrhoeae* (7), *Cwl* (fly) in *Bacillus subtilis* (10), and *iap* in *Listeria* (13), but we have not been able to find any sequence homology between these enzymes and GNA33.

The gna33 gene deletion not only perturbs the cell separation process but also seems to affect membrane architecture. By analyzing the culture supernatant of the MC58 mutant strain, we found that several proteins are specifically released in the medium. The five most abundant released proteins, unambiguously identified by MALDI-TOF mass spectrometry, belong to the outer membrane family. Moreover, four of these proteins are major outer membrane proteins of MC58. Further analysis by two-dimensional electrophoresis has allowed the identification of more than 50 membrane proteins (unpublished data). At present we can only speculate on the possible mechanism of selective membrane protein release, which can occur either because of a physical dissociation of membrane material leading to the formation of membrane vesicles or because of selective leakage (secretion) of outer membrane proteins. Shedding of membrane vesicles during the active phase of growth is known to occur in many gram-negative bacteria (12, 22, 23, 24), including *N. meningitidis* (5). Interestingly, loss of membrane material is significantly increased in the absence of a specific subset of inner membrane, periplasmic, and outer membrane proteins that share the property of strongly interacting with the murein layer and form large complexes which physically link the inner and outer membranes, maintaining envelope integrity. Among these proteins are the Braun’s (murein) lipoprotein (Lpp) and the proteins belonging the Tol-Pal system. This system includes the peptidoglycan-associated lipoprotein (PAL), a protein anchored to the outer membrane; the periplasmic protein TolB; and the inner membrane complex formed by the TolQ, TolR, and TolA proteins (15). Any mutation in *lpp* and the *tol-pal* system perturbs the outer membrane and causes the formation of OMVs (1, 2, 15). A similar phenotype might be envisaged for the ΔGNA33 mutant, reflecting the close interaction of the GNA33 protein with the murein layer.

The GNA33 knockout mutant was unable to cause bacteremia in the infant rat model even at the highest dose used. This attenuated phenotype is likely to be due to an inability of the mutant strain to multiply in the bloodstream, probably because of higher sensitivity of the bacterial cluster to complement-mediated bacterial lysis. Using insertion mutagenesis, Sun et al. (20) identified several genes essential for pathogenesis. One of the genes identified, NMB1279, is the homolog of the *E. coli* lytic transglycosylase MltB gene. However, the *Neisseria* mutant with the deletion in the NMB1279 gene that we generated exhibits a normal diplococcal phenotype and normal growth and does not release outer membrane proteins (data not shown).

In conclusion, we have shown that GNA33, a highly con-
served lipoprotein from *N. meningitidis*, is required for maintaining proper cell division, morphology, membrane architecture, and virulence of meningococci. The inability of the GNA33 mutant to cause bacteremia in infant rats suggests that GNA33 may be a good candidate for antimicrobial drugs.

**ACKNOWLEDGMENTS**

We thank Renzo Nogarotto for helpful discussions, Davide Mercati for technical assistance, Catherine Mallia for editing, and Giorgi Corsi for artwork.

J. Adu-Bobie was a recipient of a Marie Curie Fellowship from the European Union.

**REFERENCES**