INFECTION AND IMMUNITY, Nov. 1987, p. 2734–2740
0019-9567/87/112734-07$02.00/0

Vol. 55, No. 11

Molecular Cloning and Expression of *Neisseria meningitidis* Class 1 Outer Membrane Protein in *Escherichia coli* K-12

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Received 14 May 1987/Accepted 3 August 1987

A genomic library of meningococcal DNA from a clinical isolate of *Neisseria meningitidis* was constructed in the expression vector λgt11. Outer membrane complex was prepared from the same strain and used to immunize rabbits to raise polyclonal anti-outer membrane complex serum. The amplified library was probed with this polyclonal serum, and seven expressing recombinants were isolated; further investigations indicated these to be identical. The expressed meningococcal gene in these recombinants was fused to vector β-galactosidase and shown to encode epitopes present on the 42-kilodalton class 1 outer membrane protein. Estimation of the size of the recombinant fusion protein suggests that up to 40 kilodaltons of protein-coding sequence is present. The λgt11 recombinant contains a 3.4-kilobase DNA insert, which has been recloned into a plasmid and characterized by restriction endonuclease analysis. A restriction fragment from the insert, representing the protein-coding region hybridizes to a single 2.2-kilobase *Xba*I fragment from the homologous strain and to similar-sized *Xba*I fragments in other strains of meningococci, expressing antigenically distinct class 1 proteins.

The continuing incidence of life-threatening meningococcal infections in both developed and developing countries emphasizes the need for an effective vaccine against *Neisseria meningitidis*. Observations that meningococcal infection or carriage elicits protective antibodies directed against surface components such as capsule and outer membrane proteins (OMPs) (5) identify these antigens as potential vaccine candidates. Polysaccharide capsules are responsible for serogroup specificity of meningococci, and purified capsular vaccines have proven to be of some value against most meningococcal serogroups (9). However, the immunogenicity of these polysaccharides is reduced in infants (10), the age group most susceptible to infection, and the group B polysaccharide is poorly immunogenic even in adults (20), a particular problem since group B strains are the commonest cause of meningococcal infection in many countries. These problems focus attention on OMPs which elicit bactericidal antibodies during infection (4, 14) and are therefore potential alternative candidates for production of new or improved vaccines.

Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has shown the meningococcal outer membrane to contain a limited number of proteins, and on the basis of differing M_r and peptide map analysis, five major structural classes have been defined (19). Although different strains exhibit distinct OMP profiles, all meningococci possess either a class 2 or a class 3 protein as quantitatively the major protein of the outer membrane, expression of the two classes being mutually exclusive. Class 2 or 3 proteins exist as a trimer in the native membrane, forming an aqueous pore (11) which allows diffusion of essential nutrients. Despite their common function, a large degree of interstrain diversity is exhibited by these proteins, which are used to define the serotype of the organism (7).

The class 5 protein shows not only interstrain but also intrastrain heterogeneity. Different meningococcal strains each have the ability to produce a number of distinct class 5 proteins, which may occur in a variety of combinations (13), and antigenic shift in expression of class 5 protein occurs during the course of meningococcal infection (13, 18).

The antigenic heterogeneity of the two predominant OMPs of meningococci focuses attention on more conserved proteins as potential vaccine candidates. The class 4 protein, which is present in all strains in association with the class 2 or 3 porin, appears highly conserved (19). However, by analogy with the similar gonococcal protein, it is likely that anti-class 4 antibodies may block the bactericidal effect of antibodies directed against other OMPs (15). Less information is available on the biological properties of the class 1 protein which is expressed by most isolates. Although relative abundance varies between strains and in a few isolates expression appears to have been completely switched off, expression is antigenically stable within a strain. Meningococci may be divided into subtypes on the basis of immunological differences between class 1 proteins, although a considerable degree of structural similarity is exhibited (19).

Cloning of individual meningococcal outer membrane proteins in *Escherichia coli* should provide more detailed information on protein structure, interstrain variation, and possible regulatory sequences and may provide insight into functions in vivo. In addition, the production of recombinant proteins would provide invaluable material for vaccine studies.

This report describes the construction of a genomic library of meningococcal DNA in the expression vector λgt11, its screening with polyclonal anti-outer membrane complex (OMC) serum, and the detection of recombinants expressing the class 1 protein.

MATERIALS AND METHODS

Enzymes and chemicals. Restriction endonucleases, T4 DNA ligase, and calf intestinal phosphatase were obtained from Boehringer Mannheim Biochemicals and used according to the instructions of the manufacturer. T4 kinase was obtained from Bethesda Research Laboratories. Isopropyl-

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β-D-galactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside were obtained from Sigma Chemical Co. Nitrocellulose was obtained from Schleicher & Schuell, Inc., and Hybond N was obtained from Amersham International.

Bacterial strains and bacteriophage. All meningococci were clinical isolates. Strains MC50, MC51, MC52, MC54, and MC57 were obtained during 1985 from patients presenting with meningococcal meningitis by the Public Health Laboratory Service at Southampton General Hospital. Strains MC58 and MC59 were obtained from D. Jones of the Public Health Laboratory Service Meningococcal Reference Laboratory, Manchester. Isolates were cultured on proteose peptone agar and stored in liquid nitrogen (18). Subtyping was performed by D. Jones.

Restriction minus derivatives of *E. coli* strains Y1089(r^-) and Y1090(r^-) (24) were obtained from Promega Biotec; C600/λgt11 and bacteriophage λgt11 were as previously described (22). Packaging extracts were prepared as previously described (12) by using *E. coli* BHB 2688 and BHB 2690 (16). Plasmid pMTL20 is a pBR322 derivative with additional restriction endonuclease sites to pUC18 (S. P. Chambers, D. A. Barstow, and N. P. Minton, Abstr. 14th Int. Congr. Microbiol. 1986, p. 199). *E. coli* JM109 was used as a host for recombinant plasmids (21).

E. coli strains were grown in Luria broth (LB) medium (12) in liquid culture on plates supplemented with 1.5% agar (Difco Laboratories) overnight at 37°C unless otherwise described. Where stated, antibiotics were added to a final concentration of 50 μg/ml and sugars were added to 0.2% (wt/vol). DNA ligations were performed overnight at 4°C in 30 mM Tris hydrochloride, pH 7.5–10 mM $MgCl_2$–10 mM dithiothreitol–4 mM ATP with 1 U of T4 DNA ligase. Preparation and transformation of competent *E. coli* was performed as previously described (2). Recombinant bacteriophage were packaged according to methods adapted from Scalenghe et al. (16). Bacteriophage were stored at 4°C in SM buffer (12) over chloroform, and infections were performed in the same buffer. Infected cells were mixed with LB containing 0.8% agar and poured onto 2% agar LB plates containing ampicillin.

Isolation of meningococcal OMC. Meningococci were grown overnight and scraped into 0.2 M lithium acetate, pH 6.0, containing glass beads. The suspension was stirred vigorously (45°C for 2 h), cellular debris was removed by centrifugation twice (10,000 × *g* for 30 min at 4°C), and outer membranes were recovered from the supernatant by centrifugation at 100,000 × *g* for 2 h at 4°C. The pellet was suspended in 6 M urea–0.2 M sodium acetate, pH 6.0, and incubated at 25°C for 30 min, before being diluted approximately three times with distilled water and repelleted. The final pellet was suspended in water containing 1 mM phenylmethylsulfonyl fluoride and 0.05% sodium azide.

Immunological methods. Polyclonal anti-OMC serum was obtained from rabbits which were immunized subcutaneously with OMC (100 μg) in complete Freund adjuvant, followed by boosts in incomplete adjuvant on days 10 and 20. Rabbits were bled on day 25, and serum was stored at −20°C until required. Before use, cross-reacting anti-*E. coli* antibodies were removed by absorption with an *E. coli* lysate prepared from the lysogen *E. coli* C600 (λgt11). A 500-ml induced culture of the lysogen (12) was pelleted (4,000 × *g* at 4°C) and suspended in 10 ml of NP-40 buffer (50 mM Tris hydrochloride, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.25% [wt/vol] gelatin [Difco], 0.05% Nonidet P-40 [NP-40] [BDH], and 0.05% [wt/vol] NaN_3), and the cells were lysed by quick freezing in liquid nitrogen. After incubation with DNase I (20 μg/ml) for 30 min on ice, the lysate was poured onto a nitrocellulose disk and incubated for 5 min at room temperature with gentle shaking. Excess lysate was decanted, and the filter was washed with NP-40 buffer for 5 min at room temperature with shaking. A second filter was prepared in the same way, and 33 μl of serum, diluted to 5 ml in NP-40 buffer, was incubated sequentially with the two filters for 20 min with shaking at room temperature. The absorbed serum was diluted to the final working concentration of 1/300, and lysate debris was removed by centrifugation at 2,000 × *g* for 10 min at room temperature.

Rabbit antisera raised against class 1 protein which had been purified by preparative SDS-PAGE was a gift from B. Crowe, Max Planck Institute, Berlin, and was used at a concentration of 1/2,000.

Anti-β-galactosidase monoclonal antibody was obtained from Promega Biotec and used at a 1/5,000 dilution (as recommended by the manufacturer).

Affinity purification of antibodies recognizing cloned protein. Affinity purification of antibodies from polyclonal sera was performed according to Gotschlich et al. (8) with some adaptations. Phage, expressing recombinant protein, were grown at 42°C, and plaque proteins were lifted onto IPTG-impregnated nitrocellulose filters as described. The filters were blocked with bovine serum albumin and incubated with a 1/100 dilution of either anti-OMC serum or anti-class 1 protein serum for 4 h at 37°C with shaking. Unbound antibodies were removed by washing three times in NP-40 buffer, and bound antibody was eluted with 5 ml of 0.15 M glycine hydrochloride, pH 2.3, for 15 min. The eluate was neutralized by adding 1 M Tris, bovine serum albumin (to 2%), and phenylmethylsulfonyl fluoride (to 1 mM) and stored at −20°C in 500-μl portions. Eluted serum was used without further dilution in Western blots (immunoblots).

SDS-PAGE. Proteins for analysis by SDS-PAGE were separated either on linear gradients (10 to 25%) of polyacrylamide (acrylamide:bis; 51:1.3% [wt/vol]) with 4% stacking gels for 20 h at 4°C at 200 V or on 10 or 7.5% linear gels with 4% stacking gel for 4 to 6 h at room temperature at 150 V.

OMC was loaded on gel tracks (2 μg per track), and optimal amounts for loading of *E. coli*-phage lysate proteins were determined on analytical gels. Samples were mixed with equal volumes of dissociating buffer and heated to 100°C for 5 to 10 min before being loaded. Proteins were visualized by staining with PAGE-blue (BDH).

Western blotting. Proteins separated by SDS-PAGE were electrophoretically transferred to nitrocellulose membranes at 25 V and 4°C for at least 16 h in 20 mM Tris–150 mM glycine–0.1% SDS–20% methanol. Membranes were washed (four times, 15 min each) in 50 mM Tris hydrochloride, pH 7.4–150 mM NaCl and blocked in the same buffer containing 3% (wt/vol) bovine serum albumin and 0.5% (wt/vol) sodium azide for 1 h at 37°C.

Proteins from phage plaques were transferred to nitrocellulose by growing phage at 42°C until plaque size reached 0.3 mm in diameter (2.5 to 3 h). Nitrocellulose disks, previously soaked in IPTG and air dried, were placed over the plaques, incubated in situ at 37°C for a further 3 h, and blocked with bovine serum albumin.

All nitrocellulose filters were rinsed briefly in NP-40 buffer and incubated with antibody diluted in NP-40 buffer at predetermined concentrations for 1 h at room temperature. Unbound antibody was removed by rinsing in NP-40 buffer, and bound antibody was detected by using ^{125}I-labeled staphylococcal protein A, diluted to 10^6 cpm/ml. Excess

protein A was removed by washing in NP-40 buffer or sarcosyl buffer (containing 0.4% sodium *n*-lauryl sarcosine in place of NP-40 and with 1 M NaCl). Bound radioactivity was detected by fluorography at −70°C using Eastman Kodak X-Omat/X-AR5 film with a Du Pont Cronex Lightning-Plus intensifying screen for 1 to 7 days.

Construction of meningococcal genomic library in λgt11. DNA was extracted from strain MC50 as described by Tinsley and Heckels (18). DNA (2.5 μg) was digested with *Eco*RI and ligated to dephosphorylated λgt11 arms (1 μg) in 5 μl (total volume). The ligation mixture was packaged in vitro by using freeze-thaw lysate and sonicated extracts prepared from *E. coli* BHB 2688 and BHB 2690, respectively (12).

Phage-competent *E. coli* Y1090(r^-) were prepared from a 10-ml LB overnight culture containing 0.2% maltose. Cells were harvested and suspended in 4 ml of 10 mM $MgSO_4$. Packaged phage were plated onto Y1090(r^-) and amplified to obtain a high-titer stock.

Production of recombinant protein by lysogenized *E. coli*. Phage-competent *E. coli* Y1089(r^-) were obtained by growth in LB containing 100 mM Tris hydrochloride, pH 7.5, 10 mM $MgCl_2$, and 0.2% maltose. Cells were diluted 1 in 10^3 to 10^4 in LB containing 10 mM $MgCl_2$, and a portion (5 μl) was infected with 95 μl of phage stock, obtained by suspending a single picked plaque in 1 ml of SM buffer. Infected cells were incubated at 30°C for 30 min, inoculated onto 1.5% agar plates containing ampicillin, and incubated overnight at 30°C. The resulting single colonies were replica picked onto two plates; one was then incubated overnight at 42°C, and the other was incubated at 30°C. Colonies which grew only at the permissive temperature were selected as lysogens.

Lysogen proteins were obtained by inoculating 50 ml of LB with 1 ml of an overnight culture of lysogen. Cultures were incubated at 30 to 32°C until the optical density at 550 nm reached 0.5, induced at 45°C for 15 min, and then incubated at 40°C for 3 h after the addition of IPTG to 10 mM. Cells were harvested, suspended in phosphate-buffered saline (3 ml), and sonicated until the solution was no longer viscous. The sonicated lysates were stored at −20°C with phenylmethylsulfonyl fluoride added to 1 mM.

Preparation of phage DNA. Recombinant λA1 phage DNA was prepared from two 500-ml cultures of the induced lysogen (12). After DNase treatment, the cellular debris was removed by centrifugation at 12,000 × *g* for 15 min, and an equal volume of SM containing 20% (wt/vol) PEG 6000 and 1 M NaCl was added to the supernatant. Phage were precipitated at 4°C overnight and pelleted in 25-ml glass corex tubes at 12,000 × *g* for 20 min at 4°C. Drained pellets were suspended in 8 ml of SM, and cesium chloride was added to 0.75 g/ml. The solution was centrifuged at 150,000 × *g* for 24 h at 15°C in a swing-out rotor (6 by 14 ml). The phage band was harvested and dialyzed against SM buffer. Phage DNA was liberated by the addition of SDS to 0.5%, EDTA to 20 mM, and proteinase K to 50 μg/ml, followed by incubation at 65°C for 1 h. The suspension was cooled on ice, extracted with phenol-chloroform (1:1) twice and chloroform once, and precipitated with isopropanol. Suspended DNA was further purified by using an Elutip-d column (Schleicher & Schuell).

Plasmid preparation. Recombinant plasmids were prepared from *E. coli* JM109 by alkaline lysis (1), followed by NACS-52 Pre-Pac chromatography (Bethesda Research Laboratories).

Southern blotting. Restriction endonuclease digests of genomic DNA were separated on 0.6% agarose gels and transferred to Hybond N (Amersham International) as described by the manufacturer. Probe DNA was obtained by restriction endonuclease digestion, followed by agarose gel electrophoresis. Required fragments were excised from the gel and electroeluted by using a Biotrap apparatus (Schleicher & Schuell). Purified fragments were dephosphorylated and labeled with [γ-^{32}P]ATP by using T4 kinase as described by Maniatis et al. (12). Membranes were prehybridized, hybridized, and washed at 65°C according to the standard conditions described by the manufacturer.

Dried membranes were exposed to Kodak XAR-5 film at −70°C with a Cronex Lightning-Plus intensifying screen for 1 to 3 days.

RESULTS

Construction of meningococcal genomic library. *Eco*RI-digested meningococcal DNA was ligated into dephosphorylated λgt11 arms, packaged in vitro, and plated onto Y1090(r^-). Approximately 10^5 PFU were obtained per μg of DNA; 87% of plaques were white on plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside and IPTG, indicating insertional inactivation of the vector β-galactosidase by foreign DNA fragments. The library was amplified on Y1090(r^-) to obtain a high-titer stock.

Detection of recombinant bacteriophage expressing meningococcal surface antigens. OMC was prepared from strain MC50 and shown to contain four major proteins representative of classes 1, 2, 4, and 5 by SDS-PAGE (Fig. 1, track C). The complex was used to immunize rabbits. The hyperimmune serum obtained reacted with all four proteins and also with a lower-molecular-weight protein not detected by SDS-PAGE staining techniques corresponding to the pathogenic neisserial antigen (H8) on Western blots (Fig. 1, track B). This serum was also found to contain considerable anti-*E. coli* reactivity by dot blot analysis, but this was substantially removed by prior absorption with an *E. coli* lysate as described above.

To screen the library for OMP expression, Y1090(r^-) was infected with approximately 10^5 PFU of the amplified library on 15-cm-diameter petri dishes, and plaques were screened in duplicate by probing with the absorbed polyclonal serum. Many positive signals were obtained. After repicking and reprobing, seven recombinants were selected and single, well-isolated plaques from each were picked and amplified to give high-titer stocks. Restriction analysis of all seven recombinants with *Eco*RI showed them to be identical, and one recombinant, λA1, was chosen for further study.

λA1 phage was spotted onto two lawns of Y1090(r^-), and plaque proteins were lifted on two nitrocellulose filters, one of which had been soaked in IPTG. Probing the bound proteins with anti-OMC-absorbed serum gave a much stronger signal in the presence of IPTG, suggesting that the expressed protein was under the inducible control of the β-galactosidase promoter.

Characterization of the recombinant protein. To determine which of the possible OMP antigens was being expressed, antibodies which recognize the cloned protein were specifically adsorbed from the polyclonal anti-OMC serum. The serum was incubated with nitrocellulose filters, previously lifted from either λA1 recombinant or λgt11 control plaques. The eluted antibody was used to probe Western blot strips of OMC, separated on a 10% slab polyacrylamide gel. The serum eluted from λA1 derived proteins reacted with a band which appeared to comigrate with the class 1 protein. However, resolution of the class 1 and 2 proteins by this gel

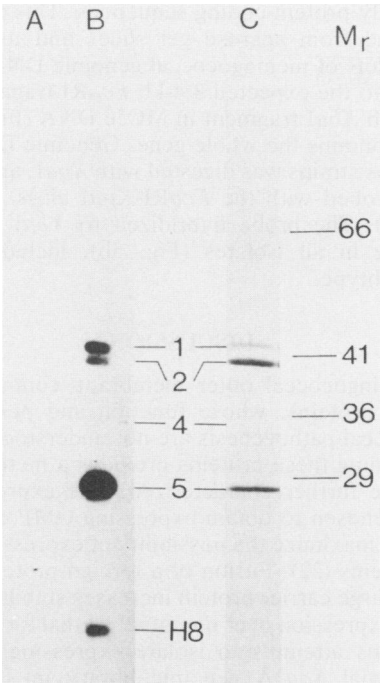

FIG. 1. OMC from MC50. Track C, PAGE-blue-stained SDS-polyacrylamide (10 to 25% gradient) separation of the OMC obtained from meningococcal strain MC50, shows representative proteins from classes 1, 2, 4, and 5. The class 4 protein is present in relatively small amounts in this preparation. Track B, Rabbit polyclonal serum (1/300 dilution) raised to this OMC was used to probe Western blots of separated MC50 OMPs. The serum reacts with the class 1, 2, 4, and 5 OMPs and a lower-M_r band, which is the H8 antigen, not visible on stained gels. Track A, Preimmunization rabbit serum shows no cross-reactivity with the meningococcal OMC. Values at right, 10^3.

system was incomplete, and hence unambiguous identification of the cloned protein was not possible. No such reactivity was detectable in the serum eluted back from λgt11-derived proteins.

A polyclonal anti-class 1 protein serum which was obtained from B. Crowe was shown to react only with the class 1 proteins of several different meningococcal isolates (unpublished data). Two meningococcal strains were chosen which possess similar- or identical-molecular-weight class 1 proteins but various class 2 or class 3 proteins by SDS-PAGE (Fig. 2a). The Western blot profile of these strains with the anti-class 1 serum confirmed the specificity of the serum for the class 1 protein (Fig. 2b). Lysogens of λA1 and λgt11 were constructed in *E. coli* Y1089(r^-), and lysates of IPTG-induced lysogen proteins were obtained. The anti-class 1 serum reacted strongly with a single high-molecular-weight band in the λA1 lysogen track, but not with the λgt11 control (Fig. 2b). The anti-class 1 polyclonal serum was also incubated with λA1-derived proteins bound to nitrocellulose. Specifically bound antibody was eluted and used to probe Western blots of the λA1 and λgt11 lysogen proteins and also meningococcal OMC. The blot gave identical results to those obtained with unadsorbed serum (Fig. 2c). Thus, these results confirm that the recombinant expressing clone λA1 contains part of the structural gene for the class 1 protein and that the protein is apparently produced as a high-molecular-weight fusion with the vector β-galactosidase.

To estimate the amount of coding sequence present in the recombinant λA1 clone, the size and nature of the fusion protein was investigated. IPTG-induced λA1 and λgt11 lysogen proteins were separated on a 7.5% polyacrylamide slab gel for 6 h, resulting in greater separation of high-molecular-weight proteins (Fig. 3a). The β-galactosidase band at 116 kilodaltons (kDa) in the λgt11 lysogen track was replaced by an approximately 156,000 M_r band in the recombinant λA1. Despite the limited resolution of protein bands in this gel system, it is estimated that up to 40 kDa of the class 1 protein is fused to the carboxy terminus of β-galactosidase in λA1. A Western blot of these proteins was probed with anti-β-galactosidase monoclonal antibody (Fig. 3b). This monoclonal antibody reacts with a band which comigrates with the putative β-galactosidase fusion protein seen in the λA1 lysogen track. In both lysogen and β-galactosidase standard tracks, a lower M_r band is seen, which is a breakdown product of β-galactosidase; the product is the same molecular weight in all tracks and reacts with the monoclonal antibody. The intensity of the breakdown product band in the recombinant track is markedly stronger than that in the λgt11 track, suggesting that the recombinant protein is inherently less stable than the native λgt11-encoded protein. Probing similar tracks with the anticlass 1 protein polyclonal serum confirmed that the high-molecular-weight band contains class 1 protein epitopes. Therefore, the

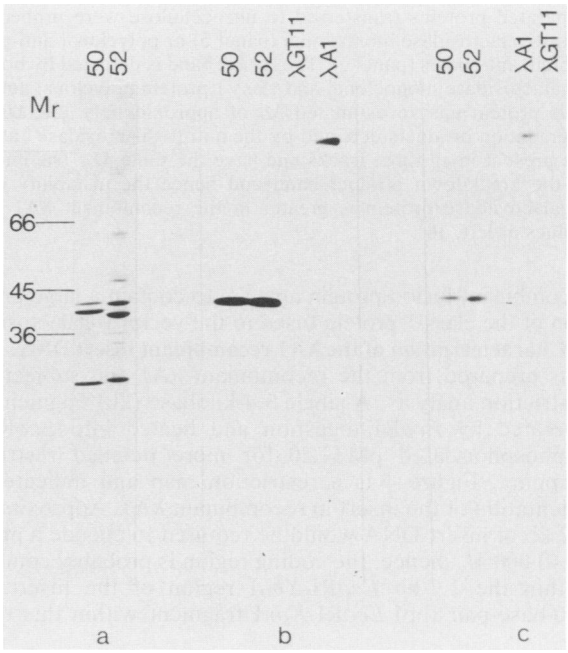

FIG. 2. λA1 is expressing MC50 class 1 protein fused to β-galactosidase. OMCs from meningococcal strains MC50 and MC52 which have similar M_r class 1 proteins but different M_r class 2 proteins (panel a) and IPTG-induced λA1 and λgt11 lysogen proteins were separated on a 10 to 25% gradient polyacrylamide gel. Western-blotted proteins were probed with anti-class 1 polyclonal serum (panel b) or antibodies from this serum, affinity purified by λA1 plaque proteins (panel c). The specificity of the antiserum for the class 1 protein is confirmed, as the serum reacts only with the similar M_r class 1 proteins. A high-molecular-weight band is detected only in the recombinant λA1 lysogen track. Antibodies from this serum are bound by λA1 plaque proteins, and these eluted antibodies produce a blot profile identical to that obtained with the unadsorbed serum. Values at left, 10^3.

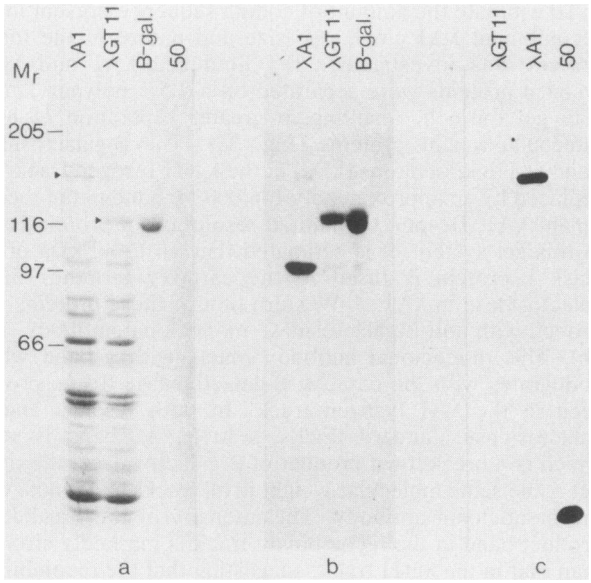

FIG. 3. Size estimation of the β-galactosidase-MC50 class 1 fusion protein. λA1- and λgt11-induced lysogen proteins and strain MC50 OMC were separated on a 7.5% polyacrylamide gel for 6 h at room temperature. The putative fusion band is clearly visible (▶) in the λA1 track and absent in the λgt11 track, which contains a band (▶) comigrating with the β-galactosidase standard (panel a). Only the class 1 and 2 OMPs from MC50 are still visible on this gel. Separated proteins transferred to nitrocellulose were probed with anti-β-galactosidase monoclonal (panel b) or polyclonal anti-class 1 protein antibodies (panel c). The fusion band is detected by both the β-galactosidase monoclonal and class 1 protein polyclonal antisera. This protein has an estimated M_r of approximately 156,000. The degradation products detected by the anti-β-galactosidase antibody are present in all three tracks and have the same M_r. The intensity of the breakdown product band and hence the instability of the β-galactosidase protein is greater in the recombinant λA1 track. Values at left, 10^3.

recombinant fusion protein appears to contain a large proportion of the class 1 protein fused to the vector β-galactosidase.

Characterization of the λA1 recombinant insert DNA. DNA was prepared from the recombinant λA1 and subjected to restriction analysis. A single 3.4-kilobase (kb) fragment was liberated by *Eco*RI digestion and ligated into *Eco*RI-cut dephosphorylated pMTL20 for more detailed restriction mapping. Figure 4 is a restriction map and indicates the orientation of the insert in recombinant λA1. Approximately 1.2 kb of insert DNA would be required to encode a protein of 40,000 M_r; hence, the coding region is probably contained within the 1.2-kb *Eco*RI-*Xba*I region of the insert. The 700-base-pair (bp) *Eco*RI-*Kpn*I fragment within this region

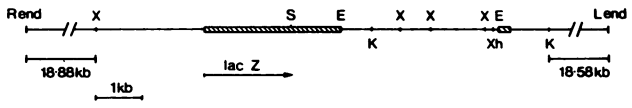

FIG. 4. Restriction endonuclease map of recombinant λA1 DNA. Symbols: ▭, β-galactosidase-coding sequences; →, direction of transcription of the β-galactosidase gene. The limits of the meningococcal DNA insert within the λgt11 vector are indicated by the *Eco*RI sites (E). Other restriction sites are *Kpn*I (K), *Xba*I (X), *Xho*I (Xh), and *Sst*I (S). The 1.2-kb *Eco*RI-*Xba*I fragment contains the expressed protein-coding sequence, and the 700-bp *Eco*RI-*Kpn*I fragment within this region was used as a hybridization probe for Southern blots.

contains only protein-coding sequences. This fragment was electroeluted from agarose gel slices and used to probe Southern blots of meningococcal genomic DNA. The probe hybridized to the expected 3.4-kb *Eco*RI fragment and to a single 2.2-kb *Xba*I fragment in MC50 DNA (Fig. 5a), which therefore contains the whole gene. Genomic DNA from six heterologous strains was digested with *Xba*I, and a Southern blot was probed with the *Eco*RI-*Kpn*I class 1 gene probe from MC50. The probe hybridized to *Xba*I fragments of similar size in all isolates (Fig. 5b), including those of different subtype.

DISCUSSION

The meningococcal outer membrane contains a limited number of proteins, whose function and possible role in meningococcal pathogenesis are not understood. Cloning of genes encoding these proteins provides a method by which they can be further characterized. The expression vector λgt11 was chosen to obtain expressing OMP clones as it is designed to maximize the possibility of expression of recombinant proteins (22). Fusion of a foreign protein gene to an inducible, large carrier protein increases stability and allows control of expression over potentially lethal foreign proteins. Our previous attempts to isolate expressing OMP clones from a partial *Sau*3A genomic library in λL47.1 failed, possibly because of adverse effects of foreign OMP expression. Meningococcal transcriptional-translational signals may also be insufficient to obtain expression of protein in *E. coli*, and this potential problem is removed by placing the gene under β-galactosidase control signals. The major OMP from *Chlamydia trachomatis* has been cloned and expressed in the λgt11 vector as a fusion protein (17).

Construction of a totally random genomic library in λgt11 statistically should allow isolation of any gene of interest. Detection of required expressing clones from an *Eco*RI

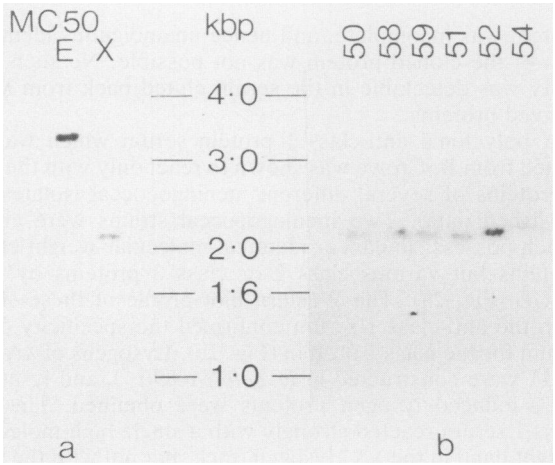

FIG. 5. The class 1 gene is localized on a single 2.2-kb *Xba*I fragment in meningococcal DNA. Genomic DNA from MC50 (panel a), digested with *Eco*RI (E) and *Xba*I (X), was probed with the 700-bp *Eco*RI-*Kpn*I fragment from the λA1 insert. The probe hybridizes to the expected 3.4-kb *Eco*RI fragment and to a single 2.2-kb *Xba*I fragment containing the entire class 1 structural gene from MC50. Genomic DNA from different meningococcal isolates was digested with *Xba*I and probed with the same *Eco*RI-*Kpn*I fragment (panel b). This probe hybridizes to similar-size *Xba*I fragments in all isolates, including at least one with a different subtype specificity to MC50. (Known subtype specificities are as follows: strains MC50, MC58, and MC59, P1.16; and MC54, P1.3.)

digest library relies on the presence of an *Eco*RI site within the gene of interest, in the correct reading frame and orientation to achieve expression of sufficient protein to be immunologically detectable. The genomic library we describe was constructed as an initial experiment before random bank construction. A good polyclonal serum was available which contains reactive components against all OMPs; thus, expression of relatively small fragments of protein should be detectable. Also, the serum is able to detect five OMPs, thus increasing the possibility of the presence of a useful *Eco*RI site within a detectable protein of interest.

The class 1 protein from MC50 has a M_r of approximately 42,000, and from the sizing data it appears that a large proportion of the structural gene for this protein is present in the recombinant λA1. The 1.2-kb *Eco*RI-*Xba*I fragment within the λA1 recombinant has a coding potential for up to 40 kDa of protein and so probably contains most of the expressed class 1 structural gene. The λA1 recombinant, used as a probe into MC50 genomic DNA digested with *Eco*RI (data not shown), hybridizes to the expected 3.4-kb fragment and to at least two other fragments with lower intensity. This could indicate partially homologous sequences within the genome or the presence of repetitive sequences within the insert DNA. When the 700-bp *Eco*RI-*Kpn*I fragment from the λA1 insert (Fig. 4) was used to probe Southern blots of *Eco*RI-digested MC50 DNA, a single band at 3.4 kb was detected, suggesting that there is a single copy of the class 1 structural gene present in the genome. Thus, repetitive sequences may be present in the flanking regions of the gene, as described for both gonococcal and meningococcal genomes (3).

A single 2.2-kb *Xba*I fragment is detected in Southern blots of *Xba*I-digested MC50 DNA by using the 700-bp *Eco*RI-*Kpn*I fragment as a hybridization probe. Thus, the entire class 1 gene must be contained within this 2.2-kb *Xba*I fragment.

The *Eco*RI-*Kpn*I probe also hybridizes to similar-size *Xba*I fragments from different meningococcal strains and the polyclonal serum, raised against denatured class 1 protein, cross-reacts with the class 1 protein from other strains. However, class 1 proteins from different isolates are not detected on Western blot by the polyclonal anti-OMC serum raised to MC50. This suggests that common regions are present in class 1 proteins but that they are not immunogenic in the native protein. This is in accord with a previous study, using peptide mapping, which suggested that conserved regions of the class 1 protein were hydrophobic and therefore probably membrane associated (19).

The class 1 proteins are candidates for inclusion in an alternative meningococcal vaccine. Antibodies to the class 1 protein are elicited during meningococcal infection (24) and may be protective against several different serotypes (6), since epitopes present on this antigen are shared by different serotype strains (25). The complete nucleotide sequence of this gene, together with further comparative studies of different meningococcal class 1 protein genes will indicate regions of the protein which are highly conserved and which may be immunogenic in man. Information on the genetic organization and control of expression of surface antigen genes will be important in the understanding of meningococcal virulence.

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