CLONING OF THE STRUCTURAL GENES OF THREE H8 ANTIGENS AND OF PROTEIN III OF *NEISSERIA GONORRHOEAE*

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The surface antigens of the gonococcus have been intensively studied in the past dozen years, and these studies have been critically reviewed (1, 2). Johnston and Gotschlich (3) found that the general architecture of the cell wall is typical for a gram-negative organism. While the outer membrane contains many protein antigens, three occur in large amounts: proteins I, II, and III (PI, PII, PIII) (4). PI is invariably expressed, is predominant in quantity (3), and is a porin acting as an aqueous channel (5–8) that is somewhat anion selective. This protein shows antigenic variation between different strains, and two major groups, PIA and PIB, are recognized (9, 10). These can be further subdivided using mAbs (11), into a number of serovars. Certain biological characteristics of gonococci are related to the type of PI that the organisms bear. For instance, strains isolated from the blood stream of patients with disseminated gonococcal infection, as a rule, are resistant to the bactericidal action of normal human serum (12), and belong to group PIA, while strains isolated from uncomplicated genitourinary disease usually belong to group PIB (13, 14).

The class of proteins referred to as PII or opacity-associated proteins (15, 16) has attracted a great deal of attention because the expression of these proteins can be turned on and off at high frequency, and antigenic variation of the protein is also seen (17, 18). Their function is not known, but they mediate intergonococcal adhesions (15, 19) and appear to favor attachment to epithelial cells (20, 21). Structural genes of members of this class of proteins have been cloned (22), allowing dissection of the events accompanying expression and antigenic variation.

PIII, first described by McDade and Johnston (23), is a protein present in all strains of gonococci examined, and in marked contrast to PI and PII, no molecular mass or antigenic variation has been noted (24, 25). Characteristically, its M_r, as measured by SDS-PAGE changes from 30,000 to 31,000 when treated with reducing agent (23). Studies employing bifunctional reagents have shown (23, 26) that PI and PIII are closely associated in the gonococcal outer membrane. It has been shown (27, 28) that IgG antibodies directed to this protein are able to interfere with the complement-mediated bactericidal action of antibodies

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directed to other surface antigens. The mechanism of the blocking effect is not understood, but it is evident that the PIII antibodies do not inhibit, but rather enhance binding of complement to the gonococcal surface (28).

Using mAbs, additional gonococcal surface antigens have been delineated. Of particular interest is the so-called H8 antigen, first described by Cannon et al. (29), because it exists in all gonococci and meningococci examined, is absent in nonpathogenic *Neisseria* species, and the mAb mediates antibody/complement-dependent bacteriolysis. This antigen is unusual because it stains poorly with the common stains used for visualization of proteins on SDS-PAGE (29, 30). It is likely that the same antigen has also been observed by Virji et al. (31) and by Zollinger et al. (32). The chemical characterization by Strittmatter and Hitchcock (33) indicating that the H8 antigen contains lipid and that it lacks aromatic and sulfur-containing amino acids may explain the odd staining behavior. Black and Cannon (34) have isolated a λ phage clone reactive with the H8 mAb, and have found that it contains 12 and 2 kb Eco RI restriction enzyme fragments of gonococcal DNA. Southern hybridization data indicated that a similar restriction pattern was observed in other strains of gonococci and meningococci.

Using the expression vector λ gt11 (35, 36) we have isolated one clone that expresses gonococcal PIII and a number of clones that express H8 antigen. When the H8-reactive clones were examined further, it became evident that they were derived from three different gonococcal genes.

Materials and Methods

Reagents and Chemicals. Glutardialdehyde-activated affinity absorbent, isopropyl β-D-thiogalactopyranoside (IPTG),[1] polynucleotide kinase, Eco RI linkers, and some restriction enzymes were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN); additional restriction enzymes and T4 ligase were obtained from New England Biolabs (Beverly, MA). Mung bean nuclease, CNBr-activated Sepharose, and Sephacryl 200 were obtained from Pharmacia Fine Chemicals (Piscataway, NJ). The enzymes were used according to the recommendations of the vendors. λ phage packaging extracts were supplied by Vector Cloning Systems (San Diego, CA). Affinity-purified goat antibody to mouse and rabbit immunoglobulins were obtained from Tago, Inc. (Burlingame, CA), and were conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) by the method of Avrameas et al. (37). Nitrocellulose BA85 was obtained from Schleicher and Schuell, Inc. (Keene, NH) and all other reagents and chemicals were purchased from Sigma Chemical Co.

Preparation of λ gt11 Vector. *E. coli* strain Y1089 lysogenized with λ gt11 was grown in 500 ml NZCYM medium (38) to an OD of 0.800 at 550 nm in a 2.8 liter Fernbach flask. The culture was heated to 44°C over an open flame, and incubated for an additional 2 h at 37°C, and the organisms were harvested by centrifugation. The cells were suspended in 10 ml of λ dilution buffer (SM solution) (38), 500 μl of chloroform and 100 μg of deoxyribonuclease were added, and the suspension was incubated for 20 min. The debris was removed by centrifugation at 2,500 *g*, and the pellet was resuspended in 5 ml of SM solution and recentrifuged. This washing step was repeated and the phage isolated from the pooled supernates were purified by sedimentation followed by flotation in solutions of CsCl (39).

Preparation of Insert DNA. Genomic DNA was prepared from gonococcal strain R10 by the method of Nakamura et al. (40). 500 μg of DNA was treated with Eco RI methylase. The DNA was sheared by sonication and then treated with mung bean nuclease to produce blunt ends. ~30 μg of this DNA was fractionated by agarose gel electrophoresis,

[1] *Abbreviations used in this paper:* IPTG, isopropyl-β-D-thiogalactopyranoside.

and the fraction corresponding to 600–2,300 bp was electroeluted. The material was ligated to Eco RI linkers that had been phosphorylated using polynucleotide kinase (38). After ligation, the DNA was subjected to extensive Eco RI digestion, and the small nucleotides were separated from the DNA by spermine precipitation (41), followed by chromatography over a 1 ml column of Sephacryl 200.

Preparation of Phage Bank. 50 ng of insert and 2 μg of vector were ligated with T4 ligase for 18 h at 16°C in a 10 μl volume. 4 μl of the ligation mixture were added to a packaging mix and titered. The bank was amplified on three large petri dishes, and was estimated to be derived from 4.6×10^5 plaques, of which 54% contained inserts as judged by lack of β-galactosidase activity.

Immunological Methods. mAb to PIII and H8 antigen were obtained from Drs. J. Swanson and P. Hitchcock, respectively (Rocky Mountain Laboratory, National Institutes of Health, Hamilton, MT). Gonococcal PI was purified as described by Blake and Gotschlich (42, 43) from strain 120176-2, and used to immunize a rabbit. The resultant serum was passed over an affinity column consisting of purified PI covalently linked to CNBr-activated Sepharose and eluted with 100 mM glycine HCl buffer, pH 2.3, containing 0.1% Triton X-100. Immunological screening was performed by allowing ~10^5 plaques to grow for 2.5 h at 42°C, overlaying with a dry nitrocellulose filter previously impregnated with 10 mM IPTG, incubating for 2 h at 37°C, and washing the filter in blocking buffer (10 mM Tris HCl, pH 7.5, containing 0.5 M NaCl and 0.5% Tween 20) three times for 10 min. The filters were incubated overnight with antiserum diluted in the blocking buffer, washed three times, incubated with alkaline phosphatase–conjugated antiimmunoglobulin for 2 h, washed three times with blocking buffer, washed once with 50 mM Trizma base with 3 mM $MgCl_2$, and then incubated with alkaline phosphatase substrate dissolved in the Trizma base buffer and incubated at 37°C (44).

The phage from plaques identified by immunological activity were purified and used to infect strain Y1089 to produce lysogens. These were induced for phage production by shifting incubation temperature to 44°C, and then induced with IPTG for antigen production, as described by Young and Davis (36). SDS-PAGE was performed on cells lysed in the SDS-containing loading buffer (45). Electrophoretic transfer to nitrocellulose or to Durapore membranes was performed according to the methods of Towbin (46), and the Western blots were probed immunologically as described above (44), or using radioactive protein A (Amersham Corp., Arlington Heights, IL). A variation of the method of Weinberger et al. (47) was used to affinity purify antibodies from rabbit serum using the products of plaques fixed to nitrocellulose. Dense lawns of plaques (~50,000) were grown on *E. coli* Y1090 at 42°C for 2.5 h, overlaid with an IPTG-containing nitrocellulose filter, and incubated for 2 h at 37°C. The filter was washed with blocking buffer as described above, allowed to react with 50 μl of serum diluted to 5 ml with blocking buffer for 4 h, washed three times with blocking buffer, and once with 150 mM NaCl. The antibodies were eluted with 5 ml of 150 mM glycine HCl, pH 2.3, for 15 min. The eluate was promptly neutralized and used for Western blots.

DNA Hybridization Analysis. DNA hybridization analyses were performed according to the methods reviewed by Meinkoth and Wahl (48), and stringent washing conditions were used (1× SSC at 68°C).

Results

The intent of this study was to clone the structural gene coding for PI of the gonococcus. The λ gt11 bank was screened with a rabbit antiserum to PI, raised by injection with purified PI of gonococcal strain 120176-2. The antibodies from this serum (2-914) were purified by affinity chromatography using PI bound to Sepharose, and were used to select 15 reactive plaques. Clones 37 and 38 were not studied further, since Eco RI digestion of the purified phages failed to release identifiable insert fragments, nor were there any immunologically active products seen in Western blots. The character of the plaques, the approximate size of the

TABLE I
Characteristics of Clones, Plaques, and Inserts in Western Blots

Clone	Plaque reactivity*	Insert size	Western product	Hybridization with insert			
				32	33	34	39
31	4+	3,000	19.7 kD	ND	ND	ND	ND
32	4+	400	Fusion	4+	–	–	–
33	4+	1,370	31 kD	–	4+	–	–
34	3+	2,780	19.7 kD	–	–	4+	2+
35	4+	1,380	19.7 kD	–	–	4+	2+
36‡	1+	1,150 and 400	Fusion	4+	–	–	–
39	4+	1,480	Fusion	–	–	2+	4+
40	2+	400	Fusion	4+	–	–	–
41	2+	200	Fusion	2+	–	–	–
42	3+	920	19.7 kD	–	–	4+	2+
43	3+	1,780	21.2 kD	–	–	2+	4+
44	1+	400	Fusion	4+	–	–	–
45	3+	?§	21.2 kD	ND	ND	ND	ND

* The degree of reactivity of the plaques was scored subjectively.
‡ This clone contained two inserts.
§ This clone did not release an insert fragment when treated with Eco RI, but produced an immunological product identical to that of clone 43.

insert, and the M_r of the reactive products seen by Western blots are listed in Table I. The gonococcal DNA inserts isolated from clones 32, 33, 35, and 39 by Eco RI digestion and preparative agarose gel electrophoresis were labeled by nick translation and used to probe Southern blots of the phage clones under stringent conditions (see Fig. 1) (48). The results are summarized in Table I. Probe 33 was found to react solely with clone 33 (data not shown). Probe 32 was found to react only with the clones containing a 400 bp insert and with clone 42 containing a 200 bp insert. Probe 35 was found to react as well with clone 34 and 42 as with itself. Probe 35 also reacted moderately with clones 39 an 43. When probe 39 was used, the reverse result was obtained; strong hybridization signals with clones 39 and 43, and weaker reactions with clones 34, 35, and 42. Restriction enzyme mapping of the inserts confirmed the relationship between clones 39 and 43, and also the relationships between clones 34, 35 and 42 (data not presented).

Hybridization analysis of DNA from strain R10 cut with various restriction enzymes indicated that each of the probes was derived from a distinct part of the genome (See Fig. 2). For instance, when one compares the hybridization pattern with Eco RI-digested genomic DNA, one finds that probe 32 reacted with a 400 bp fragment, probe 35 with a 12 kb fragment, and probe 39 with a 6.5 kb fragment. To simplify the description of the clones other than clone 33, we will subsequently refer to three classes of clones, the 32, 35, and 39 classes, each containing two or more representatives, as indicated in Table I.

Cloning of the Structural Gene of PIII. As the clones giving rise to immunoreactive plaques were analyzed, it was found that one of them (clone 33) expressed a protein that increased in M_r from 30,000 to 31,000 upon reduction with 2-ME. Since this behavior is characteristic of gonococcal PIII (23) we investigated the possibility that this protein was being expressed by this clone. When the

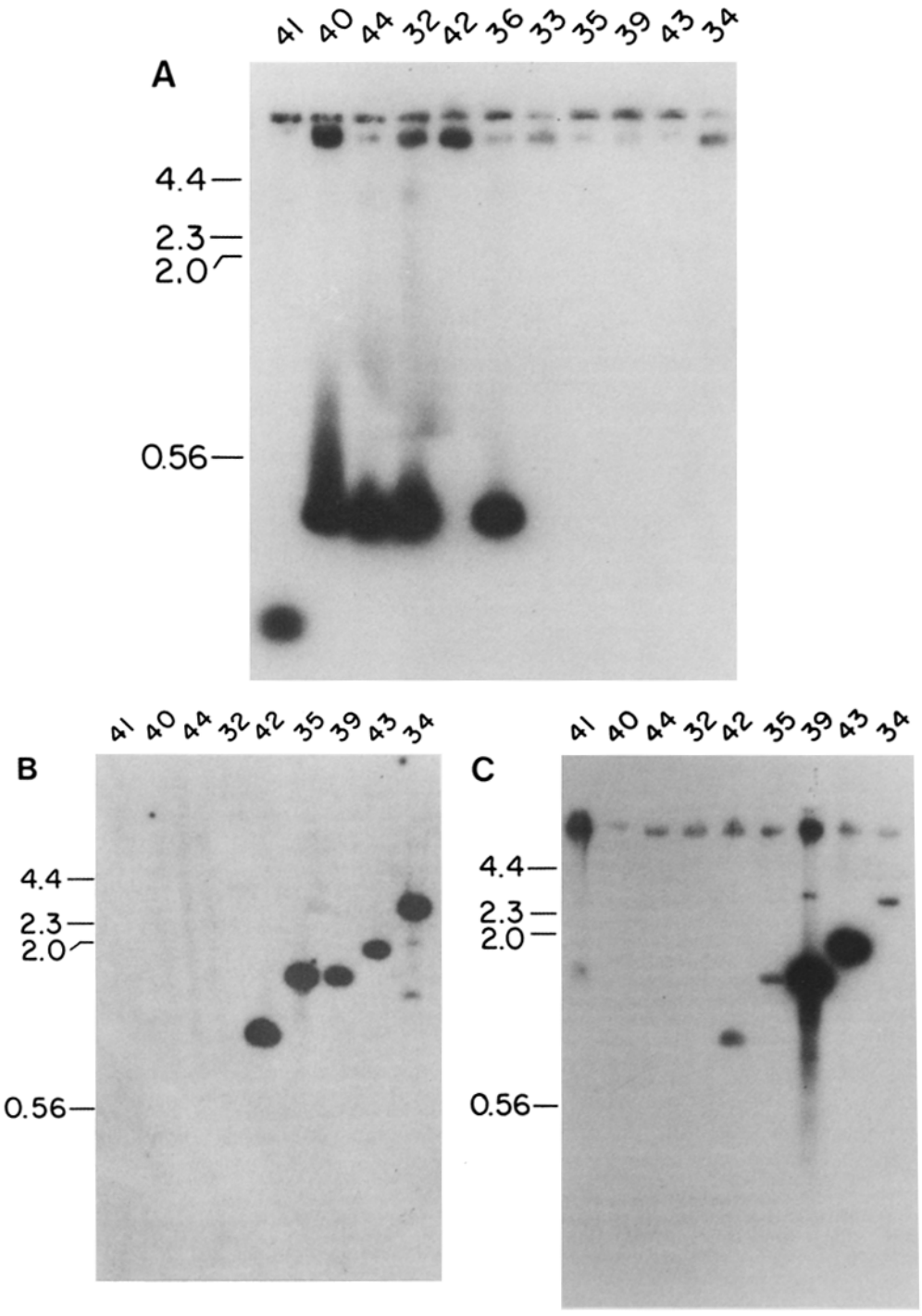

FIGURE 1. DNA hybridization of immunoreactive λ gt11 clones digested with Eco RI restriction enzyme. The numbers above the lanes refer to the number of the clone (see Table I). The positions of M_r markers are indicated on the left. The blots were hybridized with inserts from clones 32, 35, and 39 obtained by digestion with Eco RI restriction enzyme, preparative agarose gel electrophoresis, and electroelution. The inserts were labeled with ^{32}P by nick translation. *A:* Blot probed with clone 32. *B:* probed with clone 35. *C:* probed with clone 39.

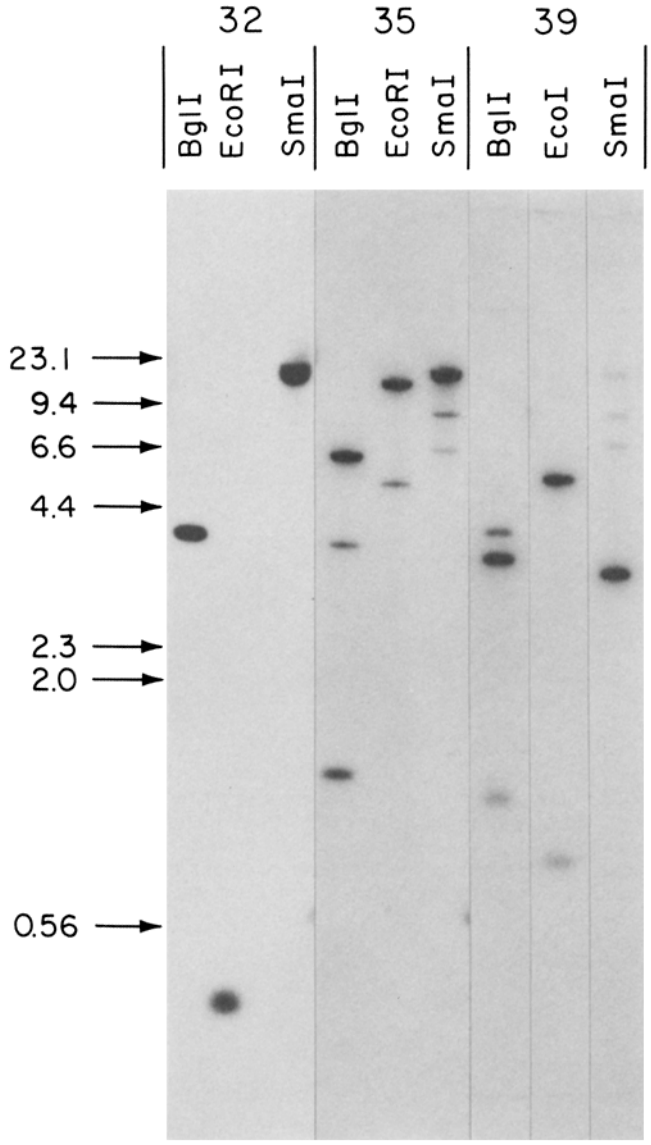

FIGURE 2. DNA hybridization of genomic DNA of gonococcal strain R10 with inserts isolated from immunoreactive λ gt11 clones. The positions of M_r markers are indicated on the left. The inserts used as probes were prepared as described in Fig. 1. Gonococcal DNA (2 μg aliquots) was digested with the restriction enzymes indicated on the figure. The probes employed are indicated at the top of the figure.

affinity-purified antiserum used for the selection of clones was examined, it was found that it did contain detectable amounts of PIII antibodies. Evidence for the nature of the product produced by clone 33 was obtained by plating ~10^5 plaques and overlaying with an IPTG-soaked nitrocellulose filter. This filter was incubated with rabbit serum containing PIII antibodies, washed, and the absorbed antibodies were eluted with acid as described above. Fig. 3 shows the reactivity

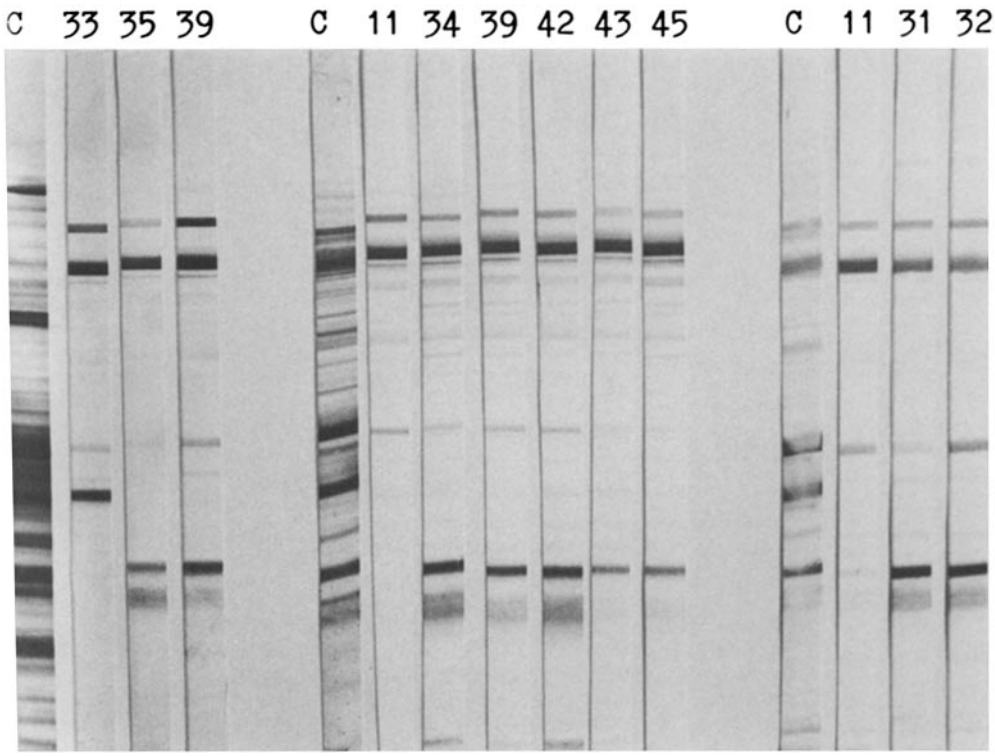

FIGURE 3. Western blot of strain R10 gonococci whole cell lysates reacted with affinity-purified antibodies derived from a rabbit antiserum to partially purified PI. The antibodies were affinity purified by letting the serum react with a dense lawn of plaques of the desired clone, and after extensive washing, eluting the bound antibodies with acid (47). The figure summarizes three separate experiments. The strips marked *C* show the reaction obtained with a 1:2,000 dilution of the antiserum. The strips marked *11* are the reactions obtained with antibodies eluted from plaques of λ gt11. The markings on the other strips refer to the number of the clone used to purify the antibody (see Table I). Clone 33 selectively absorbs PIII antibodies. The other clones absorb antibodies to two antigens with M_r of 21,200 and 19,700. Note that antibodies selected by clones 39, 43, and 45 appear to react mainly with the upper of the two bands.

of the antiserum and the eluted antibodies with an immunoblot of gonococcal proteins separated by SDS-PAGE. It is evident that the antibodies eluted from plaques of clone 33 are directed to gonococcal PIII, in contrast to those antibodies eluted from the λ gt11 plaques or the other clones.

To obtain further proof that clone 33 produces PIII, we compared the products of the clone with gonococcal PIII in an immunoblot using a PIII-reactive mAb provided by Dr. J. Swanson. It can be seen in Fig. 4 that the antibody identifies a protein that changes its mobility in SDS-PAGE depending on whether the sample has been reduced, and that the cloned product has a mobility identical to gonococcal PIII. No immunological reactivity is seen with the proteins produced by a λ gt11 lysogen. The phage containing the PIII structural gene was purified and digested with Eco RI restriction enzyme. Agarose gel electrophoresis indicated that a 1,300 bp insert was released. Thus,

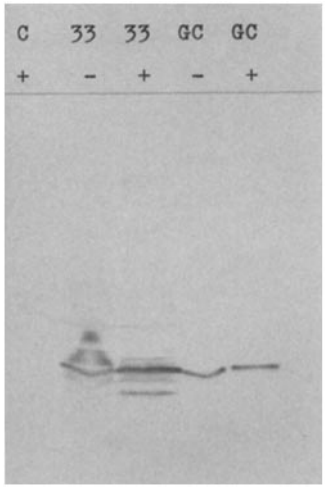

FIGURE 4. Comparison by immunoblot of the reactivity of gonococci and a lysogen of clone 33 with a PIII mAb. Alternate lanes had 2-ME (+) or no reducing agent (−) added to the sample preparation buffer. Lane *C* contains the lysate of a λ gt11 lysogen; *33* is a lysogen of clone 33; *GC* is a lysate of gonococcus strain R10. Because of the lateral diffusion of the reducing agent into the lanes not containing 2-ME, the mobility of PIII is altered at the edges, but not in the center of the lanes.

the fragment is of sufficient size to be able to contain the complete structural gene.

Clones Expressing the H8 Antigen. Nitrocellulose filters were overlaid onto dense lawns of plaques of clones 31, 32, 34, 35, 39, 42, 43, and 45, and were then used to absorb antibodies from serum 2-914, which is the serum that had been used to prepare the PI antibodies used to screen the bank. The filters were washed, and the absorbed antibodies were eluted with acid and tested by Western blots (Fig. 3) for their reactivity with gonococcal whole cell lysates. We found that, in all instances, the eluates contained antibodies to two antigens at M_r ~21,200 and 19,700 with the lower line typically quite diffuse. The character of the line and its M_r suggested the possibility that the antibodies were recognizing H8 antigen. To test this hypothesis, the reactivity of an mAb directed against the H8 antigen was compared with the acid-eluted antibodies, and the results are shown in Fig. 5. It can be seen (Fig. 5*A*) that the mAb reacts with the M_r 19,700 product of clones 31 and 35, and with the M_r 21,200 product of clone 43. While the photograph fails to show the reactivity of the fusion protein produced by clone 39, it was visible on the original autoradiograph. Fig. 5*B* shows that antibody eluted from clone 39 reacted strongly with clone 39 and 43, and crossreacted with clones 31 and 35. Fig. 5*C* shows the reverse result; antibody eluted from clone 34 reacted with clones 31 and 35, crossreacted with clones 39 and 43, and also recognized the fusion protein produced by clone 32. Fig. 5*D* shows that antibody eluted from clone 32 carrying the 400 bp insert and producing a fusion protein reacted with this clone and crossreacted with both of the other classes of clones. The three affinity-purified antibodies reacted with two antigens in the gonococcal extract. Antibodies selected with clone 34 or 32 (Fig. 5, *C* and *D*) reacted better with the lower band, while antibodies selected

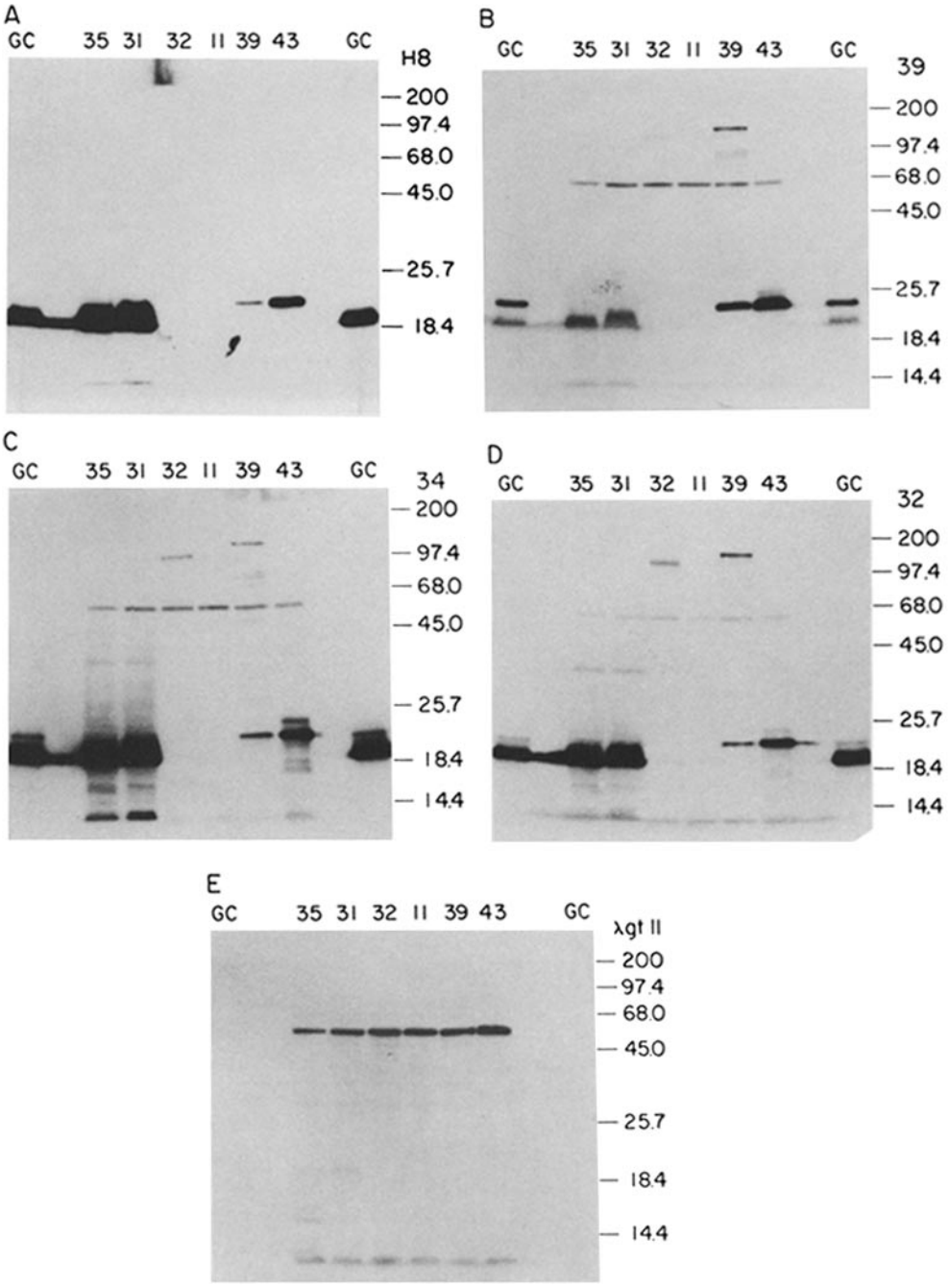

FIGURE 5. Immunoblot of selected clones and gonococcus reacted with H8 mAb or affinity-purified antibodies, as indicated in the right upper corner of each panel. The antibodies were detected by reaction with radioactive protein A and autoradiography. *A*, H8 mAb *B–E*, antibody affinity purified from plaques; *B*, clone 39; *C*, clone 34; *D*, clone 32; *E*, λ gt11. The numbers over the lanes refer to the lysogens of the clones, with *11* being a lysogen of λ gt11; *GC* refers to gonococcus strain R10. Note that in *A*, *C*, and *D* there was leakage, so that there appears to be a sample in the blank lane. Although not apparent in *A*, the original autoradiograph showed that the fusion protein produced by clone 39 reacted with H8 mAb.

with clone 39 (Fig. 5*B*) reacted more avidly with the upper band. However, the mAb (Fig. 5*A*) only recognized the smaller of these two antigens. The antibody eluted from λ gt11 did not bind to any of the products recognized by the other affinity-purified antibodies (Fig. 5*E*).

Discussion

The λ gt11 expression vector has proven to be very powerful in cloning eukaryotic genes from cDNA banks, but has been used infrequently for isolation of prokaryotic genes. Because of difficulties experienced in our laboratory in isolating the structural gene for PI in plasmids or cosmids, we decided to use λ gt11. A gene bank encompassing random genomic DNA fragments of 600–2,300 bp was obtained by established procedures using sonication to cleave DNA, mung bean nuclease to produce blunt ends, and Eco RI linkers to insert the fragments into λ gt11. Screening with two different pools of mAbs directed to PI failed to find any reactive plaques (data not given). However, screening with rabbit antiserum raised to purified PI readily identified reactive plaques. Although the affinity-purified antibody was primarily reactive with PI and had only slight reactivity with other gonococcal antigens, none of the clones selected produced PI, but clones expressing other antigens, namely PIII and H8 antigen, were selected, confirming the sensitivity and efficiency of this λ expression system.

The evidence that the structural gene of PIII has been isolated is that the clone produces as a major product a protein which is identical in M_r to gonococcal PIII, that this protein exhibits identical 2-ME-inducible shifts in M_r, that the plaques are able to selectively absorb rabbit antibody to PIII, and that the product reacts with an mAb directed to PIII. The clone contains an insert of ~1,300 bp, which is sufficient to code for the totality of PIII.

When we analyzed the remainder of the clones and the DNA of strain R10 by hybridization studies using the inserts of clones 32, 35, and 39 we found that the clones contained various fragments derived from three distinct gonococcal genes. Clones that contained a specific 400 bp insert or a fragment of this insert, and which produced immunoreactive fusion proteins, were isolated five times. Since hybridization analysis of gonococcal DNA indicated that this insert corresponded to a 400 bp genomic Eco RI fragment, it is not surprising that it was selected so frequently; it merely indicates that the protection by the Eco RI methylase reaction during the preparation of the insert was incomplete. Perhaps this explains the inability to find PI-expressing clones. Immunological evidence indicates that each of the three genes, in the context of the λ expression vector, is capable of producing protein products related to the H8 antigen. The 35 class of clones (i.e., clones 31, 34, 35, and 42) all give rise to a product of 19,700 that tends to appear as a broad band on Western blots. This material clearly reacts with H8 mAb. The clones 39 and 43 also react with the H8 mAb, and in the case of clone 43, a product of 21,200 M_r is observed. Clone 45 gave rise to the same immunological product, but Eco RI enzyme digestion did not release an insert. We assume that this clone contains a fragment of this gene, but that one of the Eco RI restriction sites is lost. It can be seen in the several panels of Fig. 5 that clone 39 gives rise to an immunoreactive fusion protein, but also to a

product indistinguishable in mobility of that of clone 43. We interpret these results to indicate that the fusion is occurring very early in the sequence of this antigen, perhaps in the signal sequence, thereby allowing a full-length processed product to appear. The evidence that the 400 bp fragment is also a part of a H8 gene is that the fusion product produced by these clones is capable of absorbing antibody that crossreacts with the products of the other H8 clones, as well as the H8 antigen in gonococci (Fig. 5*D*).

Black and Cannon (34) have obtained a λ clone that expresses H8 antigen, and they have shown that this clone contains both 12 and 2 kb Eco RI restriction fragments. We believe that it is likely that the 35 class of clones isolated in this study corresponds to the previously cloned H8 antigen, since we find that, in strain R10, it is derived from a 12 kb Eco RI fragment. The product produced by these clones is identical in mobility to the H8 antigen expressed by the parent strain of gonococcus. In addition, two other H8 antigen–related genes were found in this λ expression bank. The gene represented by the 39 class of clones is actively expressed by strain R10 of gonococcus. The evidence is that all of the affinity-purified antibodies did react with a gonococcal antigen, which had mobility (M_r of 21,200) identical to that of products produced by the 39 class of clones. This antigen is best seen with the antibody affinity-purified with clone 39. This antigen is also seen in strain 120176-2, which, in contrast to R10, is an isolate expressing a IA type of PI (data not shown). It is interesting that the mAb clearly reacts with clones 39 and 43, but fails to react with the analogous product expressed by the gonococcus. Since the reactive epitope is present in the 39 class of clones, it follows that in the analogous gonococcal product this epitope is lost or masked. The studies of Strittmatter and Hitchcock (33) showing that the H8 antigen contains lipid may be pertinent to this point. At this time little can be said about the activity of the gene from which the 32 class of clones is derived. The 32 class affinity-purified antibodies did not reveal additional H8-like antigens in the gonococcus. It is therefore not known whether this gene is actively expressed or is silent in the gonococcus. What is quite clear, however, is that the genetics and expression of the H8 antigen are more complex than had been previously realized.

Summary

A bank of gonococcal DNA was constructed in the λ gt11 expression vector. Immunological screening of the bank resulted in the isolation of a clone that contains the structural gene of protein III. In addition, several clones reactive with mAbs specific for the H8 antigen were isolated. DNA hybridization studies revealed that these H8-reactive clones were derived from three different gonococcal genes. When the products produced by these clones were used to absorb antibodies from a rabbit antiserum, and the eluted antibodies were used in immunological studies, it could be shown that the parent gonococcus expressed the product of two of these H8 genes, and in strain R10, these had M_r of ~19,700 21,200 respectively. The larger form has not been recognized hitherto because the epitope reactive with the H8 mAb may be masked in this product.

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References

1. Swanson, J. L., and L. W. Mayer. 1984. Biology of *Neisseria gonorrhoeae*. *In* Sexually Transmitted Diseases. K. K. Holmes, P. A. Mardh, P. F. Sparling, and P. Wiesner, editors. McGraw Hill, New York. 187–204.
2. Blake, M. S. 1985. Functions of the outer membrane proteins of *Neisseria gonorrhoeae*. *In* The Pathogenesis of Bacterial Infections. G. G. Jackson, and H. Thomas, editors. Springer Verlag, Berlin. 51–66.
3. Johnston, K. H., and E. C. Gotschlich. 1974. Isolation and characterization of the outer membrane of *Neisseria gonorrhoeae*. *J. Bacteriol.* 119:250.
4. Swanson, J., and J. Heckels. 1980. Proposal: nomenclature of gonococcal outer membrane proteins. *In* Genetics and Immunobiology of Pathogenic *Neisseria*. D. Danielsson, and S. Normark, editors. University of Umea, Umea, Sweden. *xxi.*
5. Greco, F., M. S. Blake, E. C. Gotschlich, and A. Mauro. 1980. Major outer membrane protein of *Neisseria gonorrhoeae* forms channels in lipid bilayer membranes. *Fed. Proc.* 39:1813.
6. Douglas, J. T., M. D. Lee, and H. Nikaido. 1981. Protein I of *Neisseria gonorrhoeae* outer membrane is a porin. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 12:305.
7. Heasley, F. A. 1980. Reconstitution and characterization of the *N. gonorrhoeae* outer membrane permeability barrier. *In* Genetics and Immunobiology of *Neisseria gonorrhoeae*. D. Danielsson, and S. Normark, editors. University of Umea, Umea, Sweden. 12–15.
8. Young, J. D.-E., M. Blake, A. Mauro, and Z. A. Cohn. 1983. Properties of the major outer membrane protein from *Neisseria gonorrhoeae* incorporated into model lipid membranes. *Proc. Natl. Acad. Sci. USA.* 80:3831.
9. Johnston, K. H., K. K. Holmes, and E. C. Gotschlich. 1976. The serological classification of *Neisseria gonorrhoeae*. I. Isolation of the outer membrane complex responsible for serotypic specificity. *J. Exp. Med.* 143:741.
10. Sandstrom, E. G., J. S. Knapp, and T. M. Buchanan. 1982. Serology of *Neisseria gonorrhoeae*: W- antigen serogrouping by coagglutination and protein I serotyping by ELISA both detect protein I antigens. *Infect. Immun.* 35:229.
11. Knapp, J. S., E. G. Sandstrom, and K. K. Holmes. 1985. Overview of epidemiological and clinical applications of auxotype/serovar classification of *Neisseria gonorrhoeae*. *In* The Pathogenic Neisseriae. G. K. Schoolnik, editor. American Society for Microbiology, Washington, D. C. 6–12.
12. Schoolnik, G. K., T. M. Buchanan, and K. K. Holmes. 1976. Gonococci causing disseminated gonococcal infection are resistant to the bactericidal action of normal human sera. *J. Clin. Invest.* 58:1163.
13. Buchanan, T. M., and J. F. Hildebrandt. 1981. Antigen-specific serotyping of *Neisseria gonorrhoeae*: characterization based upon principal outer membrane protein. *Infect. Immun.* 32:985.
14. Cannon, J. G., T. M. Buchanan, and P. F. Sparling. 1983. Confirmation of association of protein I serotype of *Neisseria gonorrhoeae* with ability to cause disseminated infection. *Infect. Immun.* 40:816.
15. Swanson, J. 1978. Studies on gonococcus infection. XII. Colony color and opacity variants of gonococci. *Infect. Immun.* 19:320.

16. Swanson, J. 1978. Studies on gonococcus infection. XIV. Cell wall protein differences among color/opacity colony variants of *N. gonorrhoeae*. *Infect. Immun.* 21:292.
17. Swanson, J., and O. Barrera. 1983. Immunological characteristics of gonococcal outer membrane protein II assessed by immunoprecipitation, immunoblotting, and coagglutination. *J. Exp. Med.* 157:1405.
18. Diaz, J. L., and J. E. Heckels. 1982. Antigenic variation of outer membrane protein II in colonial variants of *Neisseria gonorrhoeae*. *J. Gen. Microbiol.* 128:585.
19. Swanson, J., S. J. Kraus, and E. C. Gotschlich. 1971. Studies on gonococcus infection. I. Pili and zones of adhesion: their relation to gonococcal growth patterns. *J. Exp. Med.* 134:886.
20. Draper, D. L., J. F. James, G. F. Brooks, and R. L. Sweet. 1980. Comparison of virulence markers of peritoneal and fallopian tube isolates with endocervical *Neisseria gonorrhoeae* isolates from women with acute salpingitis. *Infect. Immun.* 27:882.
21. Lambden, P. R., J. E. Heckels, L. T. James, and P. J. Watt. 1979. Variations in surface protein composition associated with virulence properties in opacity types of *Neisseria gonorrhoeae*. *J. Gen. Microbiol.* 114:305.
22. Stern, A., P. Nickel, T. F. Meyer, and M. So. 1984. Opacity determinants of *Neisseria gonorrhoeae*: DNA sequence alteration associated with gene expression and chromosomal linkage to the gonococcal pilus gene. *Cell.* 37:447.
23. McDade, R. L., and K. H. Johnston. 1980. Characterization of serologically dominant outer membrane proteins of *Neisseria gonorrhoeae*. *J. Bacteriol.* 141:1183.
24. Judd, R. C. 1982. ^{125}I-Peptide mapping of protein III isolated from four strains of *Neisseria gonorrhoeae*. *Infect. Immun.* 37:622.
25. Judd, R. C. 1982. Surface peptide mapping of protein I and protein III of four strains of *Neisseria gonorrhoeae*. *Infect. Immun.* 37:632.
26. Newhall, W. J., W. D. Sawyer, and R. A. Haak. 1980. Cross-linking analysis of the outer membrane proteins of *Neisseria gonorrhoeae*. *Infect. Immun.* 28:785.
27. Rice, P. A., M. R. Tam, and M. S. Blake. 1985. Immunoglobulin G antibodies in normal human serum directed against protein III block killing of serum-resistant *Neisseria gonorrhoeae* by immune human serum. *In* The Pathogenic Neisseriae. G. K. Schoolnik, editor. American Society for Microbiology, Washington, D. C. 427–430.
28. Joiner, K. A., K. A. Warren, M. M. Frank, and P. A. Rice. 1985. Blocking immunoglobulin G enhances complement consumption and deposition on *Neisseria gonorrhoeae*. L. *In* The Pathogenic Neisseriae. G. K. Schoolnik, editor. American Society for Microbiology, Washington, D. C. 431–434.
29. Cannon, J. G., W. J. Black, I. Nachamkin, and P. W. Stewart. 1984. Monoclonal antibody that recognizes an outer membrane antigen common to pathogenic *Neisseria* species but not to most nonpathogenic *Neisseria* species. *Infect. Immun.* 43:994.
30. Hitchcock, P. J., S. F. Hayes, L. W. Mayer, W. M. Shafer, and S. L. Tessier. 1985. Analyses of gonococcal H8 antigen. Surface location, inter- and intrastrain electrophoretic heterogeneity, and unusual two-dimensional electrophoretic characteristics. *J. Exp. Med.* 162:2017.
31. Virji, M., K. Zak, and J. E. Heckels. 1985. Use of monoclonal antibodies to detect common protein antigens present in outer membranes of *Neisseria* species. *In* The Pathogenic Neisseriae. G. K. Schoolnik, editor. American Society for Microbiology, Washington, D. C. 276–280.
32. Zollinger, W. D., J. S. Ray, E. E. Moran, and R. Seid. 1985. Identification by monoclonal antibody of an antigen common to the pathogenic *Neisseria* species. *In* The Pathogenic Neisseirae. G. K. Schoolnik, editor. American Society for Microbiology, Washington, D. C. 579–584.

33. Strittmatter, W., and P. J. Hitchcock. 1986. Isolation and characterization of the gonococcal H8- antigen. *Abstr. Annu. Meet. Am. Soc. Microbiol.* B2.
34. Black, W. J., and J. G. Cannon. 1985. Cloning of the gene for the common pathogenic *Neisseria* H.8 antigen from *Neisseria gonorrhoeae. Infect. Immun.* 47:322.
35. Young, R. A., and R. W. Davis. 1983. Efficient isolation of genes by using antibody probes. *Proc. Natl. Acad. Sci. USA.* 80:1194.
36. Young, R. A., and D. W. Davis. 1983. Yeast RNA polymerase II genes: Isolation with antibody probes. *Science (Wash. DC).* 222:778.
37. Avrameas, S., T. Ternynck, and J.-L. Guesdon. 1978. Coupling of enzymes to antibodies and antigens. *Scand. J. Immunol.* 8:7.
38. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular Cloning, a Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545.
39. Davis, R. W., D. Botstein, and J. R. Roth. 1980. Advanced bacterial genetics. A manual for genetic engineering. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 80.
40. Nakamura, K., R. M. Pirtle, and M. Inouye. 1979. Homology of the gene coding for outer membrane lipoprotein within various gram-negative bacteria. *J. Bacteriol.* 137:595.
41. Hoopes, B. C., and W. R. McClure. 1981. Studies on the selectivity of DNA precipitation by spermine. *Nucleic Acids Res.* 9:5493.
42. Blake, M. S., and E. C. Gotschlich. 1982. Purification and partial characterization of the major outer membrane protein of *Neisseria gonorrhoeae. Infect. Immun.* 36:277.
43. Blake, M. S., and E. C. Gotschlich. 1984. Purification and partial characterization of the opacity-associated proteins of *Neisseria gonorrhoeae. J. Exp. Med.* 159:452.
44. Blake, M. S., K. H. Johnston, G. J. Russell-Jones, and E. C. Gotschlich. 1984. A rapid sensitive method for detection of alkaline phosphatase conjugated anti-antibodies on western blots. *Anal. Biochem.* 136:175.
45. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.
46. Towbin, H., T. Staehlin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350.
47. Weinberger, C., S. M. Hollenberg, J. M. Harmon, S. T. Brower, J. Cidlowski, E. B. Thompson, M. G. Rosenfeld, and R. M. Evans. 1985. Identification of human glucocorticoid receptor complementary DNA clones by epitope selection. *Science (Wash. DC).* 228:740.
48. Meinkoth, J., and G. Wahl. 1984. Hybridization of nucleic acids immobilized on solid supports. *Anal. Biochem.* 138:267.