Cloning of the Gene for the Common Pathogenic *Neisseria* H.8 Antigen from *Neisseria gonorrhoeae*

WILLIAM J. BLACK AND JANNE G. CANNON*

Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Received 9 July 1984/Accepted 27 September 1984

Neisseria gonorrhoeae DNA that encodes the pathogenic *Neisseria* H.8 common antigen was cloned in the lambda phage *sep6*. The recombinant phage, designated s6H.8, was detected immunologically with a monoclonal antibody that binds to the H.8 antigen. The gonococcal and s6H.8 forms of the antigen yielded identical partial proteolysis epitope maps. *Neisseria* species that did not manifest the H.8 antigen showed little or no DNA homology with s6H.8. This clone should facilitate investigation into the clinical utility of this antigen and determination of its possible function in gonococcal pathogenesis.

The genus *Neisseria* includes both commensal species and the human pathogens *Neisseria gonorrhoeae* and *Neisseria meningitidis*. The pathogens exact a high annual toll in both human suffering and health care cost (1, 21). Currently, there is no immunoprophylaxis for gonococcal or group B meningococcal disease. Efforts to produce vaccines have been confounded by the antigenic heterogeneity found among and within strains of pathogenic *Neisseria* spp. (5, 8, 10, 11, 18, 23). Few antigenic determinants common to all gonococci or meningococci or both have been reported (7, 12, 19, 25). We recently described the H.8 antigen, a conserved feature found on all pathogenic *Neisseria* spp. (gonococci and meningococci) and absent from most commensal *Neisseria* spp. (3). This protease-sensitive and surface-exposed outer membrane component is electrophoretically distinct from other previously described (8, 11, 23) proteins of the gonococcal outer membrane. We now report the cloning in a lambda phage of gonococcal DNA which encodes the H.8 antigen. The recombinant clone was detected immunologically with a monoclonal antibody that binds specifically to the H.8 antigen (3).

A library of gonococcal strain FA1090 DNA was established in the lambda phage vector *sep6* (13). Gonococcal DNA was extracted by the method of Marmur (15), partially digested (13) with the restriction endonuclease *EcoRI* (Bethesda Research Laboratories) under conditions suggested by the supplier, and size fractionated on sucrose gradients (13). Phage DNA, prepared as described elsewhere (14, 24), was also digested with *EcoRI*, and gonococcal DNA with an average size of 15 kilobases (kb) was ligated to the phage arms (13). When FA1090 DNA was digested to completion with *EcoRI*, some fragments were larger than 18.5 kb, which is the largest size fragment that can be inserted into *sep6* (13). Therefore, it was unlikely that all of the FA1090 chromosome was represented in the *sep6* library. Recombinant phage were encapsidated in vitro (9) and plated on *Escherichia coli* LE392 (13).

Phage were probed for a clone which encoded H.8 antigenic material with monoclonal antibody H.8 (McAb H.8), which has been shown to bind specifically to the H.8 antigen in both gonococci and meningococci (3). Plaques were blotted onto BA85 nitrocellulose filters (Schleicher &

FIG. 1. Western transfer of gonococcal FA1090 and s6H.8-encoded H.8 antigen. UV-irradiated (22) *E. coli* SE5000 (22) cells were either mock infected or infected with *sep6* or s6H.8. Gonococcal cells were grown as described previously (3). Samples were solubilized at 100°C for 5 min, electrophoresed in 4 to 30% sodium dodecyl sulfate-polyacrylamide gels, transferred to nitrocellulose, and probed with McAb H.8 as described previously (3). Lane 1, Radioiodinated electrophoretic molecular weight standards; lane 2, gonococcal FA1090 cells (under these solubilization conditions, native gonococcal H.8 antigen bands at both 20,000 and 30,000 daltons [3]); lane 3, irradiated, uninfected SE5000; lane 4, irradiated, *sep6*-infected SE5000; lane 5, irradiated, recombinant phage s6H.8-infected SE5000. Protein loads in lanes 3, 4, and 5 were approximately equivalent, as indicated by Coomassie blue staining (3).

* Corresponding author.

FIG. 2. Limited proteolysis and Western blotting of H.8 antigen from gonococcal strains FA1090 and s6H.8. Cell suspensions and phage lysates were diluted into sample buffer containing 0.5% sodium dodecyl sulfate and incubated at 37°C for 50 min with staphylococcal V8 protease (Miles Laboratories) by the method of Cleveland et al. (4). After electrophoresis on a 17.5% polyacrylamide gel, proteins were transferred to nitrocellulose and probed with McAb H.8 and 125 I-labeled protein A (3). Undigested H.8 antigen prepared for electrophoresis under these conditions (4) often produced a smear of antigenic material above the 20,000-dalton band, for unknown reasons. Lanes 1, 3, 5, 7, and 9, FA1090 incubated with final concentrations of 0, 2.5, 5, 10, and 20 μ g, respectively, of enzyme per ml; lanes 2, 4, 6, 8, and 10, s6H.8 digested with the same enzyme concentrations, respectively.

FIG. 3. Restriction map of s6H.8 DNA and hybridization to neisserial DNA. Restriction digests were performed as suggested by the supplier (Bethesda Research Laboratories). Nick-translated (13) s6H.8 DNA was used to probe for homology with neisserial DNA by the method of Southern (20). Phage (14, 24) and neisserial (15) DNA preparation were carried out as previously described. All reagents except 32 P-labeled triphosphates (New England Nuclear Corp.) were from Bethesda Research Laboratories. Restriction map: cos L and cos R, left and right *cos* sites of phage; R, *Eco*RI sites; H, *Hind*III sites. The two internal *Eco*RI fragments were not found in sep6. Southern transfer: MW, lambda-HindIII molecular weight

Schuell) (2), which were dried and probed with antibody and 125 I-labeled protein A (3). One clone, designated s6H.8, was found to have McAb H.8-binding material in plaques and in lysates that were cleared of cellular debris.

For confirmation that the s6H.8-associated H.8 antigenic material was phage encoded, UV-irradiated *E. coli* SE5000 cells were either mock infected or infected with sep6 or s6H.8. Strain SE5000 (22) was prepared for phage infection by growth in Luria-Bertani medium (13) plus 0.4% maltose to ca. 10^8 CFU/ml. Cells were washed, resuspended in M9 labeling medium, and irradiated ($360 \mu\text{W}/\text{cm}^2$ for 30 s) and processed as previously described (22). No viable cells were recovered after treatment. Cells were infected with phage and incubated in Luria-Bertani medium plus 10 mM MgCl_2 for 2 h before solubilization and gel electrophoresis (13). Since such irradiation destroys host cell DNA (17), antigen detected in s6H.8-infected but not in mock- or sep6-infected cells was probably s6H.8 encoded. H.8 antigenic material was found only in s6H.8-infected cells. The s6H.8-encoded H.8 antigen comigrated with the 20,000-dalton form of the gonococcal H.8 antigen on sodium dodecyl sulfate-polyacrylamide gels (Fig. 1). Under these solubilization conditions, native gonococcal H.8 antigen of strain FA1090 bands at both 20,000 and 30,000 daltons (3).

The relationship of the gonococcal antigen and the s6H.8-encoded antigen was further investigated by epitope mapping (16). FA1090 gonococcal cells and s6H.8 lysate were subjected to staphylococcal V8 protease partial proteolysis (4) and analysis by Western transfer (3) for McAb H.8-binding material. The H.8 antigenic material from the gono-

standards (23.1, 9.4, 6.6, and 4.4 kb). All other lanes contain DNA digested with *Eco*RI. ϕ , s6H.8 DNA (note that the large internal *Eco*RI fragment and the phage right arm comigrate); Ngo, *N. gonorrhoeae* FA1090 DNA; Nme, *N. meningitidis* 6625 (3) DNA; Nla, *N. lactamica* NRL30011 (3) DNA. Equivalent concentrations of each of the neisserial DNAs were used.

FIG. 4. Colony blot testing of *Neisseria* species for H.8 antigen production and DNA homology with s6H.8. (A) Colonies were blotted onto filter paper and probed with McAb H.8 as described in the text. (B) Colonies were blotted and probed by the method of Grunstein and Hogness (6) with nick-translated (13) s6H.8 DNA. Phage DNA was prepared as described previously (14, 24). *Neisseria* strains: 1090, *N. gonorrhoeae* FA1090; 6625, *N. meningitidis* 6625; 30011, *N. lactamica* NRL30011; 6865, *N. lactamica* NRL6865; 14685, *N. cinerea* NRL14685; 9300, *N. mucosa* NRL9300; 9297, *N. mucosa* NRL9297; 30009, *N. flavescens* NRL30009; 9298, *N. sicca* NRL9298; 30045, *N. sicca* NRL30045; 9993, *N. flava* NRL9993; 30017, *N. subflava* NRL30017; 14799, *N. perflava* NRL14799. Other strains: 30018, *Branhamella catarrhalis* NRL30018; 392, *E. coli* LE392. *Neisseria* and *Branhamella* strains have been described previously (3).

coccal cells and from the s6H.8 lysate gave identical monoclonal antibody-binding peptide fragments when digested with V8 protease (Fig. 2), indicating that the antigenic material produced by s6H.8 was indeed the H.8 protein.

Restriction mapping of s6H.8 DNA and Southern transfer experiments (20) confirmed that the inserted DNA in the recombinant phage was gonococcal in origin (Fig. 3). The two internal *Eco*RI fragments of s6H.8 DNA, which were 2 and 12 kb in size, were not found in sep6. DNA from s6H.8 exhibited specific homology with 2- and 12-kb *Eco*RI fragments in FA1090 DNA. Phage s6H.8 DNA also exhibited specific homology with DNA restriction fragments from two other *Neisseria* strains, both of which expressed the H.8 antigen (3) (Fig. 3).

We used a dual-colony blot format to determine whether the H.8-encoding DNA itself could be used to distinguish pathogenic from commensal *Neisseria* spp. Identical arrays of pathogenic and commensal *Neisseria* strains were blotted and probed with either McAb H.8 (3) or s6H.8 DNA (6). Species which manifested the H.8 antigen exhibited the greatest DNA homology with s6H.8 (Fig. 4). Of the strains that produced the H.8 antigen, *Neisseria cinerea* NRL14684 showed the least hybridization with s6H.8 DNA. It may be that this strain shares homology with only a portion of the s6H.8 insert DNA, including the sequences that specify the H.8 protein. Species that did not bind McAb H.8 showed little or no DNA homology with s6H.8. Phage s6H.8 DNA also showed some homology with *E. coli* LE392 DNA in this assay, presumably owing to hybridization of bacteriophage lambda sequences in the probe with LE392 DNA. Thus, the DNA sequences that specify H.8 production, as well as the other sequences on the 14-kb insert, are apparently not present in the genome of the commensal *Neisseria* spp. that do not produce the H.8 antigen.

Identification and characterization of conserved gonococcal antigens is important for both diagnosis and prophylaxis of gonococcal infection and may also contribute to understanding the mechanisms of gonococcal pathogenesis. The H.8 antigen is one of the few common surface antigens that

have been described for *N. gonorrhoeae* (3). Other common surface antigens of gonococci include OMP-MC, a protein complex of high apparent molecular weight (7), outer membrane protein III (12), and a conserved region within the protein subunits of pili (19). The cloning of DNA encoding the H.8 antigen should facilitate investigations into the potential clinical utility of the antigen and may also enable us to determine whether the H.8 antigen plays a role in neisserial pathogenesis. We have used the s6H.8-encoded H.8 antigen to demonstrate that there is a specific antibody response to the H.8 antigen in patients with disseminated gonococcal and meningococcal infection (J. R. Black, W. J. Black, and J. G. Cannon, *J. Infect. Dis.*, in press). The function of this conserved feature and its potential in immunoprophylaxis are currently being investigated.

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