Applications of Restriction Endonuclease Fingerprinting of Chromosomal DNA of Neisseria meningitidis

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Identification of microorganisms usually rests on one or more phenotypic characteristics such as morphology and biochemical or serological reactions. In this way, only a tiny fraction of the genotype of the organism is accounted for. The use of restriction endonucleases provides a novel approach, as the organization of the microbial genotype may be directly assessed (14).

The epidemiological value of genomic fingerprinting as applied on plasmid DNA from varius isolates of gram-negative bacilli has recently been described (11-13, 15). Further, in the epidemiology of herpes simplex virus this potent tool has been used successfully (3, 7). However, the potential of genomic fingerprinting for differentiation between phenotypically closely related bacteria has until recently (4, 10) remained largely unexplored.

A unique epidemiological situation prompted us to investigate the usefulness of the restriction endonuclease technique for studies of Neisseria meningitidis in northern Norway. Since 1974 this region has experienced a severe epidemic of meningococcal disease, almost exclusively caused by sulfat-resistant strains of capsular polysaccharide group B and outer membrane protein type 15 (1, 2, 8). As B15 strains have been predominant among cases of fulminant meningococcal disease and also are frequently isolated from healthy carriers, it may seem logical to assume that the clinical diversity of meningococcal infection reflects differences in host resistance rather than in bacterial virulence. However, although grouping and typing obviously have some bearing on bacterial virulence (6), more sensitive means of strain differentiation are required for further studies on the interaction between various meningococcal strains and the host organism. Hence, the purpose of this preliminary study was to gain experience with the restriction endonuclease technology for work on chromosomal bacterial DNA and, further, to look for the possible application of such genomic fingerprinting for the differentiation of similar strains of N. meningitidis.

MATERIALS AND METHODS

Bacterial strains. Included in this study were 3 non-B15 meningococci: BL/80 (group A, nontypable), ALG (non-
8.0); and 50 μl of pronase (25 mg/ml) in 50 mM Tris buffer (pH 8.0). The resulting suspension was thoroughly mixed and kept on ice for at least 1 h. For DNA extraction, 250 μl of chloroform and 250 μl of phenol saturated with water were added, and the resulting suspension was vigorously shaken until homogenized. After centrifugation for 3 min, the watery supernatant fluid and the intermediate white cap were transferred to another Eppendorf tube. This step was repeated at least four times by adding 500 μl of watery phenol only. The final DNA solution (approximately 0.5 ml) was dialyzed for at least 3 h against DNA buffer (10 mM Tris, 10 mM NaCl, pH 7.4). The final DNA concentration was measured spectrophotometrically at 260 nm and averaged 800 to 1,200 μg/ml.

Cleavage of bacterial DNA with restriction endonucleases. Twenty-five units of restriction endonuclease HindIII, EcoRI, SacI, KpnI, or XhoI (New England Biolabs, Beverly, Mass.) dissolved in buffer as recommended by the manufacturer was added to ca. 25 μg of DNA. The volumes were adjusted with distilled water to 100 μl and incubated in Eppendorf tubes for 4 h at 37°C. Finally, 10 μl of staining solution (0.05% bromphenol blue in 50% glycerol) was added to the digest.

Polyacrylamide gel electrophoresis. Electrophoresis was carried out on a vertical slab gel apparatus (Protein Dual Slab Cell with cooling, Bio-Rad Laboratories, Richmond, Calif.). The gels were prepared by mixing 10 ml of 40% acrylamide and 1.6% bis-acrylamide (Bio-Rad), 10 ml of a gel buffer stock solution, 30 ml of glycerol (87%), and 50 ml of distilled water. The mixture was deaerated under vacuum for 10 min. The gel buffer stock solution was prepared by dissolving 72.36 g of Tris base, 36.85 g of boric acid, and 6.23 g of sodium EDTA in distilled water adjusted to a volume of 1 liter. Just before casting, the gels, 60 μl of TEMED (N,N',N",N"-tetramethylethylenediamine; Bio-Rad) and 600 μl of 10% ammonium persulfate were added. Before the samples were loaded, the gels (1.5 mm thick) were prerun for 30 min at 40 mA constant current. Each cell was then loaded with 100 μl of DNA digest, and two gels were run simultaneously at 40 mA constant current until the voltage reached 580 V. From this point the gels were run at constant voltage for approximately 20 h. The temperature of the gels was kept at 10°C throughout the run.

DNA from the Escherichia coli phage P4 digested by HindIII was used as a fragment size marker (9). The gels were finally stained with ethidium bromide for 15 min, washed in distilled water for another 20 min, and photographed in UV light with a Polaroid MP-3 Land camera, using Polaroid type 55 film (Polaroid Co., Cambridge, Mass.) and a Kodak Porter professional filter no. 2B (Eastman Kodak Co., Rochester, N.Y.)

RESULTS

Choice of endonuclease and reproducibility of the method. The respective restriction patterns, or fingerprints, of one meningococcal strain after specific DNA digestion by five different restriction endonucleases varied considerably. EcoRI and HindIII both provided 50 to 60 lines of various intensities fairly well spread along the electrophoretic lane. The patterns obtained with SacI, KpnI, and XhoI appeared under the present conditions to be less suited for the detection of strain variations due to low numbers of lines or poor distribution of lines in the gel and were therefore not further explored. Cleavage with HindIII was chosen as the standard procedure. When different isolates were shown to share identical HindIII restriction patterns, identity was confirmed by EcoRI digestion.

The reproducibility of the fingerprinting method was studied by repeated testing of the DNA preparations of all meningococcal strains included. Both the technical reproducibility and the remarkable stability of the genetic organization of individual meningococcal strains were illustrated by the fact that the original HindIII genomic fingerprints were maintained in the strain (2079) submitted to 29 passages on chocolate agar. This finding was confirmed by EcoRI digestion. Also, the original restriction patterns were maintained in the strain (937/81) that was followed in vivo for 13 months.

Restriction patterns of non-B15 and B15 meningococcal carrier isolates. Figure 1 shows the respective patterns of three non-B15 and five B15 strains, all isolated from the pharynxes of healthy meningococcal carriers. As would be expected, none of the three phenotypically different strains shared identical DNA fingerprints. Interestingly, however, marked heterogeneity was also observed among the fingerprints of the five serologically identical B15 isolates. However, in all fingerprints, one single band of approximately 1.6 kilobases and a double band of approximately 1.4 kilobases were clearly visible.

Restriction endonuclease patterns of meningococcal isolates from two patients with meningococcal septicemia and their contacts. Figure 2 shows the fingerprints of two B15 meningococcal isolates recovered from the blood of two soldiers (A, B) with meningococcal septicemia and of B15 meningococcal isolates cultured from the throats of their four roommates. The fingerprints of the A and B isolates were shared by two of the carrier isolates (D and E), whereas the fingerprint of F lacked one single band of approximately 1.5 kilobases. The fourth carrier (C) harbored a B15 meningococcal possessing a fingerprint clearly different from the others.
strains. These results suggest an epidemiological potential of this technique. Preliminary investigations at our laboratory also indicate an association between certain restriction patterns and increased virulence. If this holds true, yet another dimension will be added to the use of this technique in medical microbiology.

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LITERATURE CITED


