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# Serology of *Neisseria gonorrhoeae*: W-Antigen Serogrouping by Coagglutination and Protein I Serotyping by Enzyme-Linked Immunosorbent Assay Both Detect Protein I Antigens

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A total of 224 strains were serogrouped by coagglutination (COA) and serotyped by protein I enzyme-linked immunosorbent assay (ELISA). Of these strains, 61 were from patients with disseminated gonococcal infection, 21 were from patients with pelvic inflammatory disease, and 115 were from patients with uncomplicated gonococcal infection in Singapore, the Philippines, and Denmark. Twenty-seven were laboratory reference strains. Of the patient strains, 102 belonged to COA serogroup WI, and all of the 100 strains that typed with protein I serotypes 1, 2, or 3 were in this group. Most of the strains of gonococci from the 61 patients with disseminated gonococcal infection were within this group (COA WI, 53 or 87%; protein I serotypes 1, 2, or 3, 51 or 84%). All 46 strains that were protein I serotypes 4 through 7 were also COA serogroup WII. Protein I serotypes 8 and 9 accounted for 49 (25%) of the 197 patient strains. Twenty-eight of these strains typed as COA serogroup WII, 20 typed as serogroup WIII, and 1 typed as serogroups WII and WIII. COA W serogrouping and protein I ELISA both appeared to detect antigens on the protein I molecule of the outer membrane of *Neisseria gonorrhoeae*. Protein I serotyping, which uses unboiled organisms, may generally recognize more variable and surface-exposed antigenic determinants. In contrast, COA W serogrouping, which uses boiled organisms, may recognize less exposed shared antigenic determinants in addition to variable protein I antigenic determinants. Both methods may prove useful for further studies of the epidemiology and pathogenesis of gonorrhea.

Several serological classification systems for *Neisseria gonorrhoeae*, based on carbohydrate or protein antigens, or both, have been proposed. Saccharide antigens were used recently by Maeland (20), Apicella (1), Apicella and Gagliardi (2), and Sandström and Danielsson (25). These antigens were stable during subculture in vitro, and multiple antigenic determinants were often found on a single strain of gonococci. However, the saccharide antigen classification of a gonococcus was often different for isolates from different anatomical sites from a single patient at the same time, and the serology of gonococci isolated from the same site of a single patient would frequently change, even over short time periods (12; E. Sandstrom, Ph.D. thesis, Karolinska Institute, Stockholm, 1979). To obtain a more stable marker for diagnostic, epidemiological, and genetic studies, serological classification systems based on protein antigens were developed.

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Serological classification systems based primarily on protein antigens have recently been developed by Johnston et al. (17), Wang et al. (31), Buchanan and Hildebrandt (9, 15), and Sandström and Danielsson (25). The principal protein affecting these typing systems has been referred to as the major outer membrane protein (MOMP; 17), or the principal outer membrane protein (POMP; 16), because it comprises approximately 50% of the total protein in the outer membrane protein of the gonococcus. This protein shall henceforth be referred to as protein I, as recommended and agreed to by investigators of the EMBO Workshop on Genetics and Immunobiology of Pathogenic *Neisseria*, at Hemavan, Sweden, June 1980 (28). Johnston et al. described an immunodiffusion precipitation-in-gel system, using outer membrane complexes of protein and lipopolysaccharide (LPS) of each strain, that classified gonococci into 16 different serotypes (17). These serotypes did not correlate with clinical syndromes. Using undefined formalinized whole-cell antigens in an indirect mi-

croimmunofluorescence (micro-IF) system, Wang et al. (31) classified gonococci into three separate groups with subgroups. The subgroups A2 and A3 correlated with the nutritional requirements of these strains for arginine, hypoxanthine, and uracil ($A^- H^- U^-$ auxotype) and the tendency to cause disseminated gonococcal infection (DGI) (31). Hildebrandt and Buchanan identified a protein I serotype (7122) that was correlated with strains causing DGI and with the auxotype $A^- H^- U^-$ (15). This test was based on coating purified 7122 protein I to polystyrene tubes and reacting with rabbit antisera in an enzyme-linked immunosorbent assay (ELISA) (15). Since protein I serotype antigens were expressed on the surface of gonococci, whole organisms could be used to inhibit the ELISA, allowing protein I serotyping without the need to purify membranes from each strain of gonococcus being serotyped, as required for the method of Johnston et al. (17). Furthermore, protein I serotyping correlated with serum resistance, demonstrated by Hildebrandt et al. (16). In these studies, 10 serum-resistant transformants were produced by using DNA from protein I serotype 7122 donors that were resistant to killing by normal human serum, a characteristic of nearly all strains causing DGI (26). The recipient strains were serum sensitive and of a different protein I serotype than 7122, and each of the 10 transformants also acquired the 7122 protein I ELISA serotype. More recently, Buchanan and Hildebrandt (9) extended the protein I ELISA serotyping system to nine serotype molecules. Fifteen of the 16 MOMP serotype strains described by Johnston et al. (17) were tested by ELISA against these nine serotype molecules, and 14 of the 15 were serotyped (9).

Sandström and Danielsson (24, 25) developed a W-antigen serological classification system to divide gonococci into three serogroups, using immunoelectrophoresis, immunofluorescence, and coagglutination (COA). Using absorbed sera, they demonstrated that the micro-IF and COA W serogroup systems were analogous, and that the antigens were stable during subculture in vitro and in repeated subcultures from the same patient. Also, isolates from different anatomical sites in the same patient had the same subgroup (12; Sandstrom, Ph.D. thesis, 1979). The COA serogroup WI was found to correlate with micro-IF serogroups A2 and A3 (25), and more recently with ELISA protein I serotypes 1 and 2, strains 7122 and 8658 (23). These results suggested that the COA, micro-IF, and protein I ELISA serological systems might all primarily recognize antigenic determinants on protein I. This study was undertaken to further study the relationship between the ELISA protein I serotyping and COA W serogrouping systems.

## MATERIALS AND METHODS

**Strains.** A total of 224 strains were analyzed by both the COA W serogrouping and ELISA protein I serotyping systems (Table 1). All strains were confirmed as gonococci by sugar utilization tests and kept frozen at −70°C in the *Neisseria* Reference Laboratory (NRL) until used. Strains were grown on Kellogg gonococcal medium (18) in 5% $CO_2$ at 36°C in preparation for serological testing.

**Serological classification systems. (i) Protein I serotyping by ELISA.** Protein I serotyping was performed as previously described (9). Briefly, purified protein I was coated in 2-μg/ml concentrations to polystyrene tubes (13 by 75 mm; Stockwell Scientific, Monterey Park, Calif.) or to microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) at 37°C for 5 h in 50 mM Tris, pH 8.0. These tubes or plates were then reacted with a rabbit antiserum against a purified protein I serotype antigen preparation containing less than 5% LPS and approximately 15% other proteins (9). The dilution of antiserum used produced 70 to 80% of maximal optical density development, in the absence of inhibition by organisms. The small amounts of LPS contaminating each protein I serotype preparation bound much better to Dynatech polystyrene microtiter plates than to Stockwell Scientific polystyrene tubes. Thus, a serotyping system specific for protein I antigens required the use of protein I rabbit antisera that had been previously adsorbed to remove all LPS antibody (23). This antibody was removed by passing the antiserum over an affinity column containing immobilized LPS prepared by the Westphal and Jann technique (32) from that serotype strain (see below). Gonococci in a 150-Klett unit suspension were used to inhibit optical density development in the ELISA, and a strain was considered to contain the protein I serotype molecule on its surface if 900 μl of the organism suspension produced 30 to 40% inhibition of the expected optical density, depending on the inhibition produced by the positive control strains (9). All tests were done in duplicate and by an individual not aware of any previous COA or protein I serotype results for the strains being tested.

**(ii) Preparation of affinity columns containing immobilized LPS.** LPS was obtained from each protein I serotyping strain by precipitating in 80% ethanol the fractions containing LPS from the Sepharose 6B column chromatography performed during protein I purification as previously described (9). This precipitate was then used to purify LPS, using phenol-water at 68°C by the Westphal and Jann procedure (32). These LPS preparations were free of protein when analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue. In contrast, LPS samples prepared by the Westphal procedure directly from whole gonococci were frequently contaminated with small amounts of protein and were therefore not used for the affinity columns containing LPS. To prepare the affinity columns, 2 to 4 ml of phenyl-Sepharose CL-4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) equilibrated in phosphate-buffered saline (PBS) was poured into a small column. LPS dissolved in PBS was added in amounts of 10 mg of LPS per ml of phenyl-Sepharose. This amount of LPS saturated the phenyl-Sepharose columns, and approximately 90% of the LPS remained

TABLE 1. Strains of *N. gonorrhoeae* tested by COA W serogrouping and ELISA protein I serotyping

| Strain | No. | Other identification | NRL no. | Clinical syndrome | Area | Related reference |
|---|---|---|---|---|---|---|
| Reference | | | | | | |
| MOMP types A1–W16, in order | 16 | Also used as COA reference strains | 32775–32790 | | | 12, 17, 24, 25 |
| POMP serotype | 9 | | | | | |
| 1 | | M. A. Apicella, 3853 | 7122 | | | 9, 15, 16 |
| 2 | | K. H. Johnston, W16 | 8658 | | | 9 |
| 3 | | 33 | 32790 | | | 9, 17 |
| 4 | | Mel, M | 6611 | | | 6, 7, 9–11, 14, 22 |
| 5 | | 99 | 5767 | | | 3–7, 9–11, 14, 22, 31 |
| 6 | | Black, B | 8035 | | | 9 |
| 7 | | F62 | 5766 | | | 4–7, 9–11, 14, 22, 31 |
| 8 | | J. A. Maeland, 8551, | 8038 | | | 6, 9, 10, 14, 22 |
| 9 | | M. A. Apicella | 8660 | | | 1, 2, 9, 20 |
| Micro-IF | 2 | | | | | |
| B1 | | F62 | 5293 | | | 25, 31 |
| B2 | | CDC no. 9 | 5029 | | | 25, 31 |
| Clinical isolate | 34 | | | DGI | Seattle | 19 |
| | 27 | | | DGI | Atlanta | 29 |
| | 21 | | | PID | Seattle | 8, 13 |
| | 16 | | | Uncomplicated | Singapore | J. Sng and P. Perine |
| | 38 | | | Uncomplicated | Philippines | P. Perine |
| | 61 | | | Uncomplicated | Denmark | I. Lind and L. Ødum |

TABLE 2. Reaction of COA reagents with 25 reference strains

| No. | Antiserum | Absorbed with: | Reaction with[a]: MOMP reference strain | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | W | B | G | E | R | D | V | N | H |
| 1 | W16 | 5288 + 5293 | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | – | – |
| 2 | B2 | 5288 + F | + | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | – | – |
| 3 | V15 | 5288 + 5293 | 2+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | – | – |
| 4 | 7122 | 5288 + 5293 | + | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | – | – |
| 5 | N10 | 5029 + F | – | – | – | – | – | – | – | 3+ | 3+ |
| 6 | S12 | 5029 + F | – | – | – | – | – | – | – | 3+ | 3+ |
| 7 | U14 | 5029 + F + E | – | – | – | – | – | – | – | + | 3+ |
| 8 | 5293 | 5029 | – | – | – | – | – | – | – | – | – |
| 9 | F6 | 5029 + 5288 | – | – | – | – | – | – | – | – | – |

[a] Letters refer to MOMP reference strains, and numbers are NRL reference numbers.

firmly bound to the phenyl-Sepharose, presumably due to strong noncovalent hydrophobic forces, despite repeated washing of the column with PBS. The columns were coated overnight with normal rabbit serum at 4°C. After washing with PBS, 1 to 2 ml of rabbit protein I antisera was immediately passed over the LPS column, and the antisera eluted with PBS were collected in 1-ml portions. These adsorbed antisera were tested by ELISA (9) to confirm removal of antibodies to LPS and retention of antibodies to protein I. This procedure was performed to remove LPS antibodies from antisera to each of the protein I serotypes, using columns prepared from LPS of the same strain as used to produce the protein I.

(iii) COA. Antisera were raised in rabbits against crude cell walls of *N. gonorrhoeae*, adsorbed with heterologous formalinized whole gonococci to produce W serogroup specificity as previously described (25), and stored in 0.1-ml portions at −70°C until used. Reagent staphylococci containing protein A were kindly provided by Lars Rudin, Pharmacia Diagnostics, Uppsala, Sweden. The COA reagent and organism suspensions were prepared, and the test was performed as previously described (25). Briefly, 0.1 ml of absorbed antisera was added to 1 ml of 10% reagent staphylococci in 0.15 ml of PBS, pH 7.2, incubated for 5 min at room temperature, washed, and suspended to 10 ml in the same buffer. The reagent was stored in the cold and used within 3 months. One drop of reagent was mixed with 1 drop of suspension of approximately 1% (wt/vol) boiled (10 min) whole organisms, rotated for 2 min, and read under oblique transmitted light. COA reactions on the slide were read as weak (+), moderate (2+), or strong (3+) for Table 2, and the moderate and strong reactions were considered positive. For Tables 3 through 8, patterns of significant reactivity in either the COA or protein I serotyping test were indicated simply by +.

**Polyacrylamide gel electrophoresis and Western blot.** Outer membrane protein preparations of *N. gonorrhoeae* were analyzed by SDS-polyacrylamide gel electrophoresis in slabs by the method of Maizel (21). For Western blot analysis (30), the molecules from the gel were electrophoretically eluted onto nitrocellulose paper that was then washed and reacted with rabbit antiserum. The paper was again washed and then reacted with $^{125}$I-labeled staphylococcal protein A, again washed, and used to expose an X-ray plate (Eastman Kodak Co., Rochester, N.Y.; XR plate with Dupont Cronex Hi-Plus intensifying screens). This autoradiograph indicated the specific components of the membrane protein preparation to which antibodies in the antisera were directed.

## RESULTS

Table 2 shows the reactivity of the COA reagents with MOMP (17) and POMP (9) serotype reference strains. These COA reagents showed three W serogroup reactivity patterns when rabbit antisera used to prepare the reagents were first adsorbed with formalinized whole cells from strains belonging to the two heterologous serogroups. Table 2 indicates the strains used to immunize rabbits and the strains used for adsorption of each antiserum. The MOMP and POMP reference strains are indicated by letters and Arabic numerals, respectively, and are ordered according to probable antigenic relationship, as found by Sandström and Danielsson (25). The COA reagents 1 to 4 produced WI serogroup reactivity and recognized strains W through V in the MOMP reference system, or serotypes 1 to 3 in the POMP reference system (Table 2). The COA reagents 5 to 7 produced WII serogroup reactivity, recognizing serotype reference strains N to U and 4 to 7 of the MOMP and POMP systems, respectively. The COA reagent 8 reacted with MOMP reference strain A and with POMP reference strain 8 and weakly with reference strain 9. This reagent was included since it was earlier observed that absorption of anti-F6 antisera to remove cross-reactivity between WII and WIII left strain A1 nonreactive (Sandstrom, Ph.D. thesis, 1979). Reagent 8 was nonreactive, except with a subset of Singapore strains (IIB; see Table 4) and three Danish strains. The COA reagent 9 produced WIII serogroup reactivity recognizing only serotype reference strains F and 9 of the MOMP and POMP systems, respectively (Table 2).

TABLE 2—*continued*

| Reaction with[a]: | | | | | | | Reaction with[a]: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MOMP reference strain | | | | | | | POMP reference strain | | | | | | | | |
| C | X | S | T | U | A | F | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| − | − | − | − | − | − | − | 3+ | 3+ | 3+ | − | − | − | − | − | − |
| − | − | − | − | − | − | − | 3+ | 2+ | − | − | − | − | − | − | − |
| − | − | − | − | − | − | − | 3+ | 3+ | 2+ | − | − | − | − | − | − |
| − | − | − | − | − | − | − | 3+ | 3+ | + | − | − | − | − | − | − |
| 3+ | 3+ | 2+ | + | + | − | − | − | − | − | − | 3+ | 3+ | 3+ | − | − |
| 3+ | 3+ | 3+ | 3+ | 3+ | − | − | − | − | − | 2+ | 3+ | 3+ | 3+ | − | − |
| 2+ | + | 2+ | 3+ | 3+ | − | − | − | − | − | 2+ | 2+ | 2+ | 3+ | − | − |
| − | − | − | − | − | 2+ | − | − | − | − | − | − | − | − | 2+ | + |
| − | − | − | − | − | − | 3+ | − | − | − | − | − | − | − | − | 2+ |

Initially, two sets of U.S. strains from patients with DGI (50 strains) or pelvic inflammatory disease (PID) (23 strains) were tested both by COA and by protein I ELISA, using unadsorbed rabbit antisera to protein I. Forty-six of the 50 DGI strains contained protein I serotype 1, and 45 of these were also COA serogroup WI. Similarly, 16 of the 23 PID strains contained protein I serotype 1, and 13 of these strains were also COA serogroup WI. However, 23 of the 46 DGI strains and 13 of the 16 PID strains that were protein I serotype 1 also showed reactivity with serotypes belonging to serogroup WII, suggesting that some antigens other than those reactive in the COA system were contributing to the protein I serotyping with unadsorbed rabbit antisera (23). These antisera were then passed over an affinity column to which LPS from the immunizing strain had been attached, and this selectively removed all antibody to LPS while retaining antibody to protein I (Fig. 1). These affinity column-adsorbed antisera were then used in the protein I ELISA to retest the DGI and PID strains (Tables 3 and 4). All of the 36 cross-reactions between serotypes in serogroups WI and WII were removed, suggesting that these cross-reactions were contributed by LPS antigen-antibody interactions. Consequently, all subsequent protein I ELISA serotyping was performed with LPS-affinity column-adsorbed antisera. In addition, two DGI strains that had previously serotyped with unadsorbed antisera were unreactive with the adsorbed antisera. This suggested that LPS antibodies in the unadsorbed rabbit antisera may have been responsible for the reaction with these two strains and that at least one additional protein I serotype must exist among these two strains of gonococci.

As shown in Table 3, 32 of the 34 DGI strains from Seattle were COA serogroup WI, and 31 of these 32 strains were protein I serotype 1, 2, or 1 and 2, and one was nontypable. The two COA group WII strains from Seattle both had a serotype 5 and 7 pattern. Of the 27 strains from

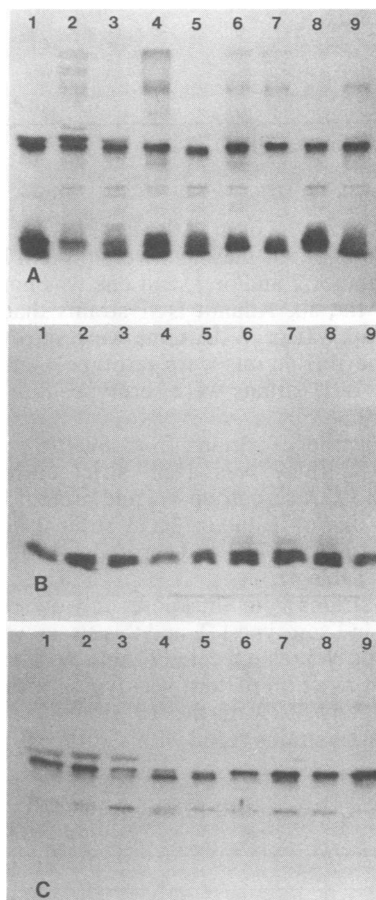


FIG. 1. Partially purified protein I preparations from each of the nine serotypes were electrophoresed over a 12.5% polyacrylamide–0.1% SDS gel, followed by electrophoresis of the protein and LPS molecules on nitrocellulose sheets (Western blot). These sheets were then washed with buffer and reacted with unadsorbed rabbit antiserum to serotype 1 protein I (A), rabbit antiserum to LPS from the prototype strain for serotype 1 (NRL 7122) (B), and the antiserum in (A) adsorbed over an LPS affinity column to remove antibodies to LPS (C).

TABLE 3. Protein I ELISA serotype and COA W serogroup results for *N. gonorrhoeae* from patients with DGI

| Area | Serogroup | | | | | | | | | | | | No. of strains |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | I | II | III | |
| Seattle | + | | | | | | | | | + | | | 4 |
| | | + | | | | | | | | + | | | 1 |
| | + | + | | | | | | | | + | | | 26 |
| | NT[a] | | | | | | | | | + | | | 1 |
| | | | | | + | | + | | | | + | | 2 |
| | | | | | | | | | | | | | 34 |
| Atlanta | + | | | | | | | | | + | | | 9 |
| | + | + | | | | | | | | + | | | 9 |
| | + | | + | | | | | | | + | | | 1 |
| | + | + | + | | | | | | | + | | | 1 |
| | NT | | | | | | | | | + | | | 1 |
| | | | | + | | | | | | | + | | 1 |
| | | | | + | + | | | | | | + | | 1 |
| | | | | | + | | | | | | + | | 1 |
| | | | | | | | | + | + | | | + | 1 |
| | | | | | | | | | + | | | + | 2 |
| | | | | | | | | | | | | | 27 |

[a] NT, Nontypable.

patients with DGI in Atlanta, 21 were COA serogroup WI. Of these, 20 were protein I serotype 1 and/or 2 and/or 3, and one was nontypable. Of the six Atlanta DGI strains that were serogroup WII or WIII, none were serotypes 1 to 3. The WII strains were serotype 4 and/or 5, and the WIII strains were serotypes 8 and/or 9 (Table 3).

Among the 21 strains from Seattle patients with PID, there was again strict correlation between COA serogroup WI and protein I serotypes 1 and/or 2 and/or 3 (13 strains) and between serogroup WII and serotypes 4 to 9 (nine strains; Table 4).

Of 16 strains from Singapore, only one reacted as protein I serotype 1,2, and this strain was also serogroup WI, as expected (Table 5). The most common reaction pattern was typing with serotypes 8 and 9 (9 of the 16 strains), and an additional 3 strains typed with serotype 8. Of the 12 serotype 8 reactive strains, eight reacted only with COA reagent 8 that reacted primarily with serotype 8 prototype strain and weakly with serotype 9 prototype strain. This pattern was classified as COA group WIIB. Six of these eight strains had protein I serotype patterns of 8,9, and two of these strains typed with only serotype 8.

Twelve of the 38 isolates from the Philippines contained serotype 1 protein I antigen and were also COA serogroup WI (Table 6). Of the 16 serogroup WII reactive strains, nine contained serotype 5 antigen. Six of these had a protein I serotype 4,5 pattern, two had a serotype 5,6 pattern, and one contained both serotype 5 and 7 antigens. Four strains typed only with protein I serotype 7, and three typed only with serotype 8 (Table 6). Ten strains reacted with serogroup WIII reagent, and six of these had a protein I serotyping pattern of 8,9.

TABLE 4. Protein I ELISA serotype and COA W serogroup results for *N. gonorrhoeae* from patients with PID

| Serogroup | | | | | | | | | | | | No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | I | II | III | |
| + | | | | | | | | | + | | | 3 |
| + | + | | | | | | | | + | | | 5 |
| + | | + | | | | | | | + | | | 1 |
| + | + | + | | | | | | | + | | | 3 |
| | | | + | + | | | | | | + | | 2 |
| | | | | + | | | | | | + | | 3 |
| | | | | + | | + | | | | + | | 1 |
| | | | | | + | | | | | + | | 1 |
| | | | | | | | + | + | | + | | 2 |
| | | | | | | | | | | | | 21 |

TABLE 5. Protein I ELISA serotype and COA W serogroup results for *N. gonorrhoeae* from patients in Singapore

| Reaction with: | | | | | | | | | | | | | No. of strains |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| POMP serotype reagent | | | | | | | | | COA W reagent | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | I | IIA | IIB | III | |
| + | + | | | | | | | | + | | | | 1 |
| | | | | + | | | | | | + | | | 1 |
| | | | | + | | + | | | | + | | | 1 |
| | | | | | | + | + | | | + | | | 1 |
| | | | | | | | + | | | | + | | 2 |
| | | | | | | | + | + | | | + | | 6 |
| | | | | | | | | + | | | + | | 1 |
| | | | | | | | + | + | | | | + | 3 |
| | | | | | | | | | | | | | 16 |

Among the 61 strains from Denmark, 24 were COA serogroup WI only, 32 were serogroup WII only, and only 5 were serogroup WIII. By protein I serotyping, all of the 24 serogroup WI strains were within serotypes 1 to 3, with the commonest pattern being serotype 1,2 (17 strains; Table 7). For the serogroup WII strains, the most common protein I serotype patterns were 8 alone (10 strains), 6 alone (5 strains), and 7 alone (4 strains). Of the six strains that were serotypes 8 and 9, three were classified as serogroup WII, and three were classified as serogroup WIII (Table 7).

Table 8 summarizes the 101 strains of this study that reacted as COA serogroup WI in terms of their reactivity pattern with specific COA reagents 1 to 4 and by protein I serotypes. One pattern of reactivity with COA reagents (pattern E; Table 8) accounted for 63% (33 of 52) of the DGI strains, as compared with 0% (0 of 12; $P < 0.001$) and 25% (6 of 24; $P < 0.001$) of strains from patients with localized infections in the Philippines and Denmark, respectively. This E pattern of reactivity was more common for the DGI isolates from Seattle patients than from Atlanta patients (27 of 31 [87%] versus 6 of 21 [29%]; $P < 0.001$), suggesting that most DGI strains in Seattle may have disseminated from a single clone of gonococci, a hypothesis supported by the fact that 26 of these 27 isolates also required arginine, hypoxanthine, and uracil for growth ($A^- H^- U^-$ auxotype; 19). In contrast, nearly half of the DGI isolates from Atlanta had an H pattern of reactivity with the COA reagents, a rare pattern for the Seattle DGI isolates (Table 8). These two COA reactivity patterns were not reflected in the serotyping patterns for protein I.

## DISCUSSION

The results in Tables 1 to 7 indicate that both the COA W serogrouping method and the principle outer membrane protein serotyping system each recognize primarily, if not exclusively, antigenic determinants on protein I. All of 100 strains that were protein I serotypes 1 and/or 2 and/or 3 were also exclusively COA serogroup WI, and all of 48 strains that were serotype 4 and/or 5 and/or 6 and/or 7 were exclusively COA

TABLE 6. Protein I ELISA serotype and COA W serogroup results for *N. gonorrhoeae* from patients in the Philippines

| Reaction with: | | | | | | | | | | | | No. of strains |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| POMP serotype reagent | | | | | | | | | COA W reagent | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | I | II | III | |
| + | | | | | | | | | + | | | 3 |
| + | + | | | | | | | | + | | | 9 |
| | | | + | + | | | | | | + | | 6 |
| | | | | + | + | | | | | + | | 2 |
| | | | | + | | + | | | | + | | 1 |
| | | | | | | + | | | | + | | 4 |
| | | | | | | | + | | | + | | 3 |
| | | | | | | | + | + | | + | + | 1 |
| | | | | | | | + | + | | | + | 5 |
| | | | | | | | | + | | | + | 4 |
| | | | | | | | | | | | | 38 |

TABLE 7. Protein I ELISA serotype and COA W serogroup results for *N. gonorrhoeae* from patients in Denmark

| Reaction with: | | | | | | | | | | | | No. of strains |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| POMP serotype reagent | | | | | | | | | COA W reagent | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | I | II | III | |
| + | | | | | | | | | + | | | 1 |
| + | + | | | | | | | | + | | | 17 |
| | + | | | | | | | | + | | | 4 |
| + | | + | | | | | | | + | | | 1 |
| | | + | | | | | | | + | | | 1 |
| | | | + | | | | | | | + | | 3 |
| | | | + | + | + | | | + | | + | | 1 |
| | | | | + | | | | | | + | | 3 |
| | | | | + | + | | | | | + | | 2 |
| | | | | + | + | + | | | | + | | 1 |
| | | | | | + | | | | | + | | 5 |
| | | | | | | + | | | | + | | 4 |
| | | | | | | | + | | | + | | 10 |
| | | | | | | | + | + | | + | | 3 |
| | | | | | | | + | | | | + | 1 |
| | | | | | | | + | + | | | + | 3 |
| | | | | | | | | + | | | + | 1 |
| | | | | | | | | | | | | 61 |

serogroup WII. The only variations between protein I serotypes and COA W serogroups were with respect to serotypes 8 and 9. Of the 16 strains that were protein I serotype 8 only, 15 were COA serogroup WII and one was COA serogroup WIII. Of the eight strains that were protein I serotype 9 only, five were COA serogroup WIII and one was serogroup WII. Twenty-four strains contained both 8 and 9 protein I serotype antigens. Eleven of these strains were COA serogroup WII, 12 were serogroup WIII, and 1 strain was classified as both serogroups WII and WIII. The discrepancies between serogroups WII and WIII and protein I serotypes 8 and 9 probably reflect differences in the antigenic determinants of protein I recognized by the separate reagents. This explanation appears more likely than an explanation that the absorbed COA reagent 9 recognizes antigens other than protein I antigenic determinants, since considerable correlation still exists between COA W serogroup reactivity and protein I serotype patterns of 8 alone or 9 alone.

It is likely that individual protein I molecules contain multiple antigenic determinants and that some of these determinants are shared and some differ between different protein I serotypes. The results in Tables 2 to 7 suggest that protein I of serotypes 1 and 2 and serotypes 8 and 9 often share antigenic determinants, whereas serotypes 4 to 7 less often share determinants. Extremely few, if any, antigenic determinants are shared between the COA serogroup WI (serotypes 1 to 3) and the group of serotypes 4 to 9 (COA groups WII and WIII). These data are consistent with the chymotrypsin peptide map data of Swanson (27) and our own (E. G. Sandstrom, K. C. S. Chen, and T. M. Buchanan) unpublished trypsin peptide maps, suggesting that there are two major biochemical groups of gonococcal protein I molecules. One group corresponds to the WI COA group and protein I serotypes 1 to 3, and the other corresponds to COA groups WII and WIII and protein I serotypes 4 to 9. Common peptides are found among all the different protein I molecules from MOMP, POMP, and micro-IF reference strains classified as COA serogroup WI, or among the different protein I molecules of strains belonging to serogroups WII and WIII. However, few, if any, peptides found within COA group WI are shared with either COA group WII or WIII. It is possible that the common peptides within COA serogroup WI, or within COA serogroups WII and WIII, represent antigens that are recognized by COA but less so by protein I serotyping by ELISA. Protein I serotyping, which uses unboiled organisms, may generally recognize the more variable and surface-exposed antigenic determinants. In contrast, COA W serogrouping, which uses boiled organisms, may recognize less exposed shared antigenic determinants in addition to variable protein I antigenic determinants. Thus, the COA serogroups WI and WII/III recognize two different groups of protein I molecules, and the ELISA protein I serotyping characterizes one or more antigenic determinants on these molecules. In view of the combined biochemical and antigenic data, it appears likely that COA group WIII represents a prominent

TABLE 8. Comparison of COA W-reagent patterns with protein I ELISA serotype for 101 COA WI strains of *N. gonorrhoeae*

| Pattern | COA serogroup WI reaction | | | | | | | | | | POMP serotype reaction | | | | | | | No. of strains |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | COA reagent[a] | | | | No. of reacting strains[b] | | | | | | Single | | | Multiple | | | | |
| | 1 | 2 | 3 | 4 | S | A | PID | Sing | Phil | Denm | 1 | 2 | 3 | 1,2 | 1,3 | 1,2,3 | NT[c] | |
| A | − | + | − | − | − | 1 | − | − | − | − | − | − | − | − | − | − | 1 | 1 |
| B | − | − | − | + | 1 | 2 | − | − | − | − | 1 | − | − | 2 | − | − | − | 3 |
| C | + | − | − | + | − | 1 | 1 | − | − | 2 | 1 (1)[d] | 1 | − | − | 1 | 1 | − | 4(1) |
| D | − | − | + | + | 1 | 1 | − | − | − | − | − | − | − | 2 | − | − | − | 2 |
| E | + | − | + | + | 27 | 6 | 4 | − | − | 6 | 7 (4) | 2 (1) | − | 30 (24) | 2 (1) | 1 | 1 (1) | 43 (3) |
| F | + | + | + | − | − | − | − | − | 1 | − | 1 | − | − | − | − | − | − | 1 |
| G | − | + | + | + | − | − | − | − | 1 | − | − | − | − | 1 | − | − | − | 1 |
| H | + | + | + | + | 2 | 10 | 7 | 1 | 10 | 16 | 11 (7) | 2 | 1 | 30 (5) | − | 2 (1) | − | 46 (13) |
| | | | | | 31 | 21 | 12 | 1 | 12 | 24 | 21 | 5 | 1 | 65 | 3 | 4 | 2 | 101 |

[a] COA reagents 1 to 4 refer to the reagents prepared as indicated in Table 2. All 9 reagents were tested on all of the 224 strains in this study; all strains reactive with any or all of the reagents 1 to 4 were classified as WI, and those reactive with reagents 5 to 7 or 8 and 9 were classified as WII or WIII, respectively.

[b] S, Seattle; A, Atlanta; Sing, Singapore; Phil, Philippines; Denm, Denmark.

[c] Nontypable.

[d] Reaction numbers for DGI strains in parentheses.

variable or "exposed" serotype protein I antigen (analogous to serotype 9), rather than a distinct third group of protein I molecules (Tables 2 to 7). It seems possible to fuse and refine these two classifications into one system with the aid of an analysis of the variable and surface-exposed tryptic peptides (Sandstrom and Buchanan, unpublished data).

Data presented previously (23) and in Table 2 indicate that both COA W serogrouping and protein I serotyping correlate significantly with strains capable of causing DGI. In addition, gonococci requiring arginine, hypoxanthine, and uracil for growth are almost invariably COA serogroup WI and protein I serotypes 1 and/or 2 and/or 3. Further unpublished studies from our laboratory indicate significant correlations of the COA W serogrouping and protein I serotyping patterns with geographic area, penicillinase production, sensitivity to killing by normal human serum, and asymptomatic gonococcal infection. It is likely, therefore, that both COA W serogrouping and protein I serotyping will prove useful methods for further studies of the epidemiology of, the pathogenesis of, and immunity to gonorrhea.

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