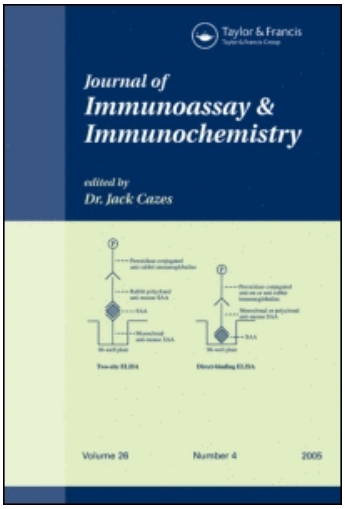

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DETECTION OF *NEISSERIA GONORRHOEAE* BY DOT-ENZYME IMMUNOASSAY USING MONOCLONAL ANTIBODIES

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ABSTRACT

A highly sensitive and specific dot-enzyme immunoassay for the detection of *Neisseria gonorrhoeae* was developed using a pool of monoclonal antibodies (MAbs). The MAbs were obtained following immunization of mice with lithium acetate extracted outer membrane (OM) preparations. Western immunoblotting experiments demonstrated that MAbs NG26 and NG38, both IgG_{2a}, reacted with lipopolysaccharides (LPS) and with the major OM protein, P1, respectively. MAb NG28, an IgG_3, did not react in Western immunoblotting. MAbs NG28 and NG38 failed to react with OM treated with proteolytic enzymes or with semi-purified preparation of LPS. MAb NG26 reacted with the same LPS preparation. Binding radioimmunoassay with live bacteria showed that all the MAbs adsorbed to cell surface-exposed antigenic determinants. The limit of

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detection of the dot-enzyme immunoassay was between 1 and 4 x 10^4 cfu per dot. Using a panel of 177 strains of *N. gonorrhoeae,* MAbs NG28 and NG38 recognized only P1A and P1B strains respectively. MAb NG26 reacted with P1A, P1B and non-typable strains. These MAbs did not react with other *Neisseria* species or other bacterial species. Using this pool, the dot-enzyme immunoassay had a sensitivity of 93.2% and a specificity of 100%. (KEY WORDS: *Neisseria gonorrhoeae*, monoclonal antibodies, dot-enzyme immunoassay.)

INTRODUCTION

Neisseria gonorrhoeae is one of the most commonly occurring sexually transmitted pathogens (1). Its detection in women is often difficult. It is also well established that asymptomatic infections may lead to pelvic inflammatory disease, ectopic pregnancy and infertility (1). Laboratory mis-identification of gram-negative diplococci as *N. gonorrhoeae* could have serious social, medico-legal and clinical implications. The laboratory must therefore use a fast and sensitive technique to accurately and rapidly identify this microorganism. Acid reaction from sugars and nitrate reduction are the standards to which other methods of culture confirmation are compared. However, these methods are occasionally compromised by limited or unpredictable growth of this fastidious bacterium (2). Substrate utilization tests depending on the presence of preformed enzymes are largely independent of bacterial growth and provide faster results, but they often require subculture of isolates. Several immunological procedures, including coagglutination with polyclonal or murine monoclonal antibodies and fluorescein or

enzyme-conjugated monoclonal antibodies, are generally confirmatory on primary or secondary culture. There is a need to improve the sensitivity and specificity of these tests. Direct antigen detection would also improve the rapidity of diagnosis.

The subject of this report is the production and characterization of monoclonal antibodies (MAbs) specific to *N. gonorrhoeae* designed for direct antigen detection. We report the use of a mixture of these MAbs in a Dot-enzyme immunoassay for rapid detection of this microorganism.

MATERIAL AND METHODS

Bacterial Strains and Culture Conditions

A total of 177 *Neisseria gonorrhoeae*, 39 *N. meningitidis*, 4 *N. lactamica*, 3 *N. mucosa*, 2 *N. cinerea*, 6 *N. perflava/sicca*, 1 *N. flava*, 1 *N. flavescens* and 4 *Branhamella catarrhalis* were obtained from the culture collections of the National Reference Centre for Neisseria and from the Antimicrobials and Molecular Biology Division of the Laboratory Centre for Disease Control, Ottawa, Canada. Other bacterial species such as *Escherichia coli*, *Haemophilus influenzae*, *Staphylococcus aureus*, *S. epidermidis* and Streptococcus A were obtained from our own collection. Strains were grown at 37ºC with 5% CO_2 on blood agar plates or on gonococcus (GC) medium supplemented with IsoVitalex (BBL, Microbiology Systems Cockeysville, Md.). They were confirmed by Gram stain, oxidase test, and carbohydrate

utilization patterns employing modified cysteine and trypticase agar (CTA; BBL). The strains were also confirmed by coagglutination tests using monoclonal antibodies (GonoGen; New Horizons Diagnostics, Columbia, Md., Solgun; Syva Diagnostics, Palo Alto, CA., Pharmacia; Uppsala, Sweden).

Outer Membrane and Lipopolysaccharides Preparations

The extraction of outer membrane (OM) from the bacteria was performed as described previously (3) with modifications. Briefly, whole cells were suspended in 300 mM lithium acetate buffer (pH 5.8) with 20 mM sodium chloride and 10 mM EDTA and shaken with 6mm glass beads at 300 rpm for 2 h at 45ºC (Environmental shaker, New Brunswick Scientific Co. Inc., New Brunswick, NJ). Cells and debris were removed by centrifugation at 15 000 x g for 20 min. The supernatant was collected and centrifuged at 40 000 x g for 2 h. The pellet was washed three times and then suspended in distilled water. The protein content was determined by the method of Lowry (4) with modifications for membrane extracts (5). Aliquots of each preparations were stored at -70ºC

Gonococcal lipopolysaccharide (LPS) was prepared by a phenol-water microadaptation of the extraction procedure described by Lambden and Heckels (6) with modifications. LPS was extracted from two strains: B2, a P1A strain and T13, a P1B strain. Briefly, bacterial cells (2 or 3 plates) were suspended in 10 ml PBS containing 0.15 mM $CaCl_2$ and 0.5 mM $MgCl_2$ and washed once in the same buffer. The pellet was suspended in 5 ml distilled water to which an equal volume of warm 90% v/v phenol was added. The suspension was stirred vigorously at 70ºC for 15 min, then cooled on ice for 10 min. The aqueous phase was removed and the remaining material

re-extracted with an equal volume of water. After centrifugation the aqueous phases were pooled and extensively dialysed against distilled water. To ensure complete precipitation of the LPS, two volumes of acetone and a few crystals of $MgCl_2$ were added to the dialysed fraction and incubated overnight at 4°C. After centrifugation, the pellet was resuspended in distilled water. This LPS preparation was used to test monoclonal antibodies.

Immunization of Mice

Balb/c mice were injected intraperitonally and in the foot pads with 10 µg of OM of strain T13 (serotype 4, P1B strain) or 20 µg of OM of strain W16 (serotype 3, P1A strain) in PBS without adjuvant. On day 7 the mice were reinjected intraperitonally with 10 or 20 µg of the same preparation. Mice were trial bled 7 to 10 days after the boost and their sera tested by ELISA and immunoblotting. On day 20 to 25, 3 days before fusion, a booster dose of 15 or 20 µg of OM in PBS was given intravenously.

Fusion Procedure

The fusion procedure was performed as described by Brodeur et al. (7). As soon as possible, usually on day 10 to 12, supernatants of wells containing growing clones were tested for MAbs directed against homologous outer membrane antigens using direct ELISA. In order to select only *N. gonorrhoeae*-specific hybridomas they were also tested against *N. meningitidis* outer membrane preparations. Selected antibody-producing cells were recloned by limiting dilution, and ascitic fluid was produced as described previously (8,9). Usually, 3.2 x 10^6 hybridoma cells were

injected intraperitonally per F1 mouse (Balb/c x Swiss Webster) pretreated with 0.5 ml pristane.

Enzyme-Linked Immunosorbent Assay (ELISA) Procedure

Supernatants of wells containing growing clones were screened by ELISA for the presence of antibodies specific to GC, using alkaline phosphatase-conjugated goat anti-mouse Ig (Sigma), as described previously (10). Briefly, 0.1 ml of a solution of antigen (OM from different strains) 5 μg/ml in 0.05 M carbonate buffer pH 9.6 was dispensed to each well in a flat-bottomed microtitration plate (Linbro, Flow Laboratories, McLean, VA) and absorbed overnight at 4°C. The microplate was washed 3 times with PBS [NaCl 137 mM, KH_2PO_4 1.5mM, Na_2HPO_4 9mM, KCl 3mM in double distilled water, final concentration 0.15M, pH 7.2-7.4] containing 0.02% Tween 20 (Sigma), and blocked with 0.2 ml of 1% bovine serum albumin (BSA) in coating buffer per well. After 30 min at room temperature the BSA was discarded. Each well received 0.1 ml culture supernatant and the plate was incubated for 60 min at 37°C. The supernatant was then removed and the plate was washed 3 times as before. Alkaline phosphatase-conjugated goat anti-mouse immunoglobulin was diluted in PBS containing 3% BSA, and 0.1 ml of this solution was added to each well. After incubating for 60 min at 37°C, the plate was washed as above and 0.1 ml of a solution of enzyme substrate, p-nitrophenyl phosphate (Sigma), 1g/L in 10% diethanolamine buffer, pH 9.8 was added. The enzyme reaction was performed at room temperature and read at 410 nm after 30 and 60 min with a Dynatech microplate Reader MR 600.

The first screening was performed using homologous OM (same strain as for immunization) and *N. meningitidis* OM in order to exclude any cross-reacting MAb. Then, to identify

MAbs directed against different *N. gonorrhoeae* strains, each positive hybrid clone supernatant was further tested against a panel of 8 gonococcus reference strains representing serotypes 1 to 9 (P1A strains: B2, serotype 1; E5, serotype 1,2; W16, serotype 3; P1B strains: T13, serotype 4; N10, serotype 5,6; H8, serotype 5,6,7; A1, serotype 8 and F6, serotype 9a,9b) and 3 *N. meningitidis* strains (representing serogroups A, B and C).

Determination of Ig Class

The class, subclass and light chain specificity of the murine Ig were determined by ELISA using commercially available reagents (Bio-Rad Laboratories, Richmond, CA). Purified rabbit anti-mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3, IgM, IgA, κ and λ chain specific anti-sera were used as described by the manufacturer in an indirect assay.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoresis of OM proteins was performed using the Bio-Rad Mini Protean system according to the method of Laemmli (11). The gels were stained with Coomassie blue and then destained as described by Weber and Osborn (12) or were transferred onto nitrocellulose paper for immunoblotting. The following proteins (Pharmacia) were used as molecular weight standards: phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), α-lactalbumin (14.4 kDa).

Immunoblotting Procedure

After SDS-PAGE, the proteins were transferred electrophoretically from the gel to nitrocellulose paper (Bio-

Rad) by the method of Towbin et al. (13). The nitrocellulose was blocked with either 1% milk (Carnation) or 1% (w/v) BSA-PBS (Sigma). The blots were incubated overnight at 4ºC with culture supernatants or 1 h at 37ºC with ascitic fluids diluted in the blocking buffer. Mouse hyperimmune sera diluted in 5% (w/v) skimmed milk were used as controls. The blots were then soaked in 5% skimmed milk containing ^{125}I-labelled goat anti-mouse Ig (Amersham, Arlington Heights, IL, 0.25 μCi=9.25 kBq) and exposed to Dupont Cronex film (Wilmington, DE).

Dot Enzyme Immunoassay

The reactivity of MAbs to a large number of bacterial strains was performed using a dot-enzyme immunoassay. All the steps in this assay were performed at room temperature. The bacteria were grown overnight on chocolate agar plates. The microbial lawns were removed from the surface of the agar plate with dacron swabs and suspended in Tris buffered saline (TBS), pH 7.5. For each strain the cell suspension was adjusted spectrophotometrically to $5x10^6$ colony-forming units (cfu)/ml ($O.D._{550nm}$ ~0.03). Dots were prepared in the Bio-Dot, microfiltration apparatus (Bio-Rad).

Fifty μl per well of each bacterial suspension was filtered through the nitrocellulose by gravity. Each well was washed twice by vacuum with 200μl of 0.02% (v/v) Tween-TBS and 200 μl of 0.5% (w/v) skimmed milk-TBS was used for blocking. The sheets were then removed from the apparatus, washed twice with Tween-TBS and soaked in a TBS solution containing 1% (w/v) skimmed milk for 30 min to block the nitrocellulose sheets. They were then incubated 1 h on a rocker platform with undiluted culture supernatant or ascitic fluid diluted 1:1000 in 1% (w/v) skimmed milk. The sheets were

washed twice in TBS and incubated for 1 hour with peroxidase-conjugated goat anti-mouse Ig (Cappel, Cooper Biomedical, Malvern, PA). After three washes, the blots were revealed in a solution of o-dianisidine prepared as described by Towbin et al. (13). The limit of detection was determined by dotting serial dilutions of two bacterial suspensions (strains B2 and T13) and incubating these with MAbs NG28 or NG38.

Adsorption of MAbs

Adsorption of MAbs at the surface of live bacteria was performed according to Martin et al. (14). The bacteria were grown overnight on chocolate agar plates, harvested with dacron swabs and suspended in PBS. The O.D. was adjusted in order to obtain a dense suspension of approximately 10^9 cfu/ml. Aliquots of 1 ml were centrifuged and the supernatant discarded. One ml of hybridoma culture supernatant was mixed with the cells and incubated for 2 hours at 4°C with constant agitation. After three washes in PBS, the bacterial cells were incubated in 1% (w/v) BSA/PBS solution containing ^{125}I-labelled sheep anti-mouse Ig (Amersham) 0.25µCi/ml for 1 h at room temperature with constant agitation. The bacteria were washed twice in PBS, and the ^{125}I counts per minute were detected using a 1282 Compugamma (LKB Instruments Inc., Rockville, MD). Bacterial counts before and after the adsorption with MAbs reflected no loss of viability.

As controls, a non-specific *N. gonorrhoeae* strain and *Haemophilus influenzae* cells were also incubated with the MAb.

Enzymatic digestion of OM

The method described by Martin et al. (14) was used to treat OM and LPS preparations with proteolytic enzymes.

Briefly, 25 μg of proteinase K (Boehringer, Mannheim, FRG) or 150 μg of protease from *Streptomyces griseus* type VI (Sigma) in PBS, pH 7.2, was mixed with 15 μg of protein of gonococcus OM preparation. After incubation for 15 min at 37ºC, the OM protein concentration was adjusted to 5 μg/ml with coating buffer, pH 9.6, and aliquots of 100μl of that suspension were placed into a Linbro E.I.A. microtitration plate or filtered through nitrocellulose paper using the Bio-Dot microfiltration apparatus (Bio-Rad). Ascitic fluid from each clone was reacted with enzymatically treated and control OM preparations and with enzymatically treated and control LPS preparations.

RESULTS

Production and Characterization of *Neisseria gonorrhoeae*-Specific MAbs

Sera from mice immunized with gonococcus OM preparations without adjuvant showed higher titers and better specificity than mice immunized with Freund's adjuvant (data not shown).

Five hybridomas, MAbs NG20, NG26, NG28, NG30 and NG38 secreting high levels of MAbs and showing different patterns of reactivity in ELISA were selected for further characterization. MAbs NG20, NG26 and NG28 were obtained after fusion of spleen cells from a mouse immunized with W16 OM (strain exhibiting OM protein 1A) and MAbs NG30 and NG38 were obtained after fusion of spleen cells from a mouse immunized with T13 OM (strain exhibiting OM P1B).

MAb NG28 reacted with 3 out of 8 reference strains, all of them P1A strains. MAbs NG30 and NG38 reacted with 5 out of 8 gonococcus reference strains, representing only strains with P1B. MAbs NG20 and NG26 reacted with 5 out of 8 gonococcus reference strains, two P1A and three P1B strains. None of the MAbs reacted with *N. meningitidis* in ELISA (Table 1).

Each hybridoma was cloned at least twice by limiting dilution, and the class and subclass of the MAb secreted were determined by ELISA using the Bio-Rad Mouse Typer Sub-Isotyping Kit. MAbs NG26, NG30 and NG38 were characterized as IgG_{2a}, MAb NG20 as an IgG_1 and MAb NG28 as an IgG_3. All the MAbs exhibited κ light chains.

Localization of Antibody-Specific Epitopes

The Western immunoblotting technique was used to determine the specific OM antigens to which the MAb bound. Mice hyperimmune sera against OM preparations were used as positive controls. Figure 1 lane a shows a serum obtained from a mouse immunized with strain B2 OM (P1A) reacting with B2 OM. Figure 1 lane g shows a serum obtained from a mouse immunized with strain T13 OM (P1B) reacting with T13 OM. In both cases we can see 5 to 6 major bands of apparent molecular weights ranging from 80 kDa to 14 kDa, including the P1 at 36-37 kDa. Two MAbs, NG30 and NG38, reacted with a OM protein of strain T13 (Figure 1 lane e and lane f respectively), while two others, MAbs NG20 and NG26, recognized epitopes present on the LPS of strain B2 (Figure 1 lane b and lane c respectively). The major OM protein, Protein 1 (P1, about 36 kDa), was identified by MAbs NG30 and NG38. A large band at the bottom of the gel, characteristic of LPS, was

Table 1

Reactivity of the MAbs against OM Preparations.

Organism	Strain	Serotype	Monoclonal Antibodies				
			NG20	NG26	NG28	NG30	NG38
Neisseria gonorrhoeae	P1A B2	1	+	+	+	0	0
	E5	1,2	+	+	+	0	0
	W16	3	0	0	+	0	0
	P1B T13	4	0	0	0	+	+
	N10	5,6	+	+	0	+	+
	H8	5,6,7	+	+	0	+	+
	A1	8	0	0	0	+	+
	F6	9a,9b	+	+	0	+	+
Neisseria meningitidis	604	A	0	0	0	0	0
	80-165	B	0	0	0	0	0
	85-060	C	0	0	0	0	0

recognized by MAbs NG20 and NG26. A band of apparent molecular weight of 38 kDa was also identified by these MAbs.

A dot-enzyme immunoassay using OM and LPS preparations digested with proteolytic enzymes was also used to confirm the immunoblotting results. MAbs NG20 and NG26 reacted with both treated and untreated OM as well as treated

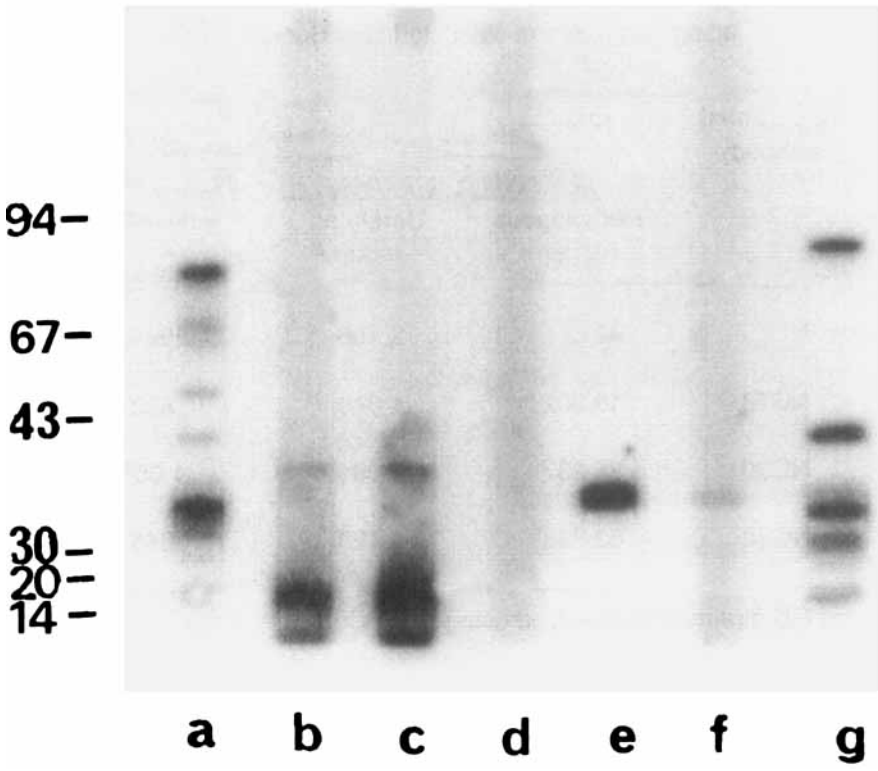

FIGURE 1. Autoradiogram of the immunoblots of strain B2 OM preparation (lanes a to d) and strain T13 OM preparation (lanes e to g) after probing with MAbs NG20 (lane b), NG26 (lane c), NG28 (lane d), NG30 (lane e) and NG38 (lane f), and mice hyperimmune sera (lanes a and g). Standard proteins are identified with their kDa values on the left.

and untreated purified LPS, confirming their reactivity with epitopes present on the LPS. MAbs NG28, NG30 and NG38 reacted only with untreated OM. Although MAb NG28 did not react in immunoblotting (Figure 1 lane d), the protein nature of the determinant recognized by this MAb was suggested by its reaction with untreated OM only.

Table 2

Binding Properties of MAbs to Intact Bacterial Cells.

Monoclonal antibody	^{125}I-labeled anti-mouse Ig (cpm) bound		
	Neisseria gonorrhoeae		*Haemophilus influenzae*
	Homologous strain	Unrelated strain [a]	
NG20	42 695	2 106	984
NG28	19 302	1 846	833
NG30	85 237	2 064	1 605
NG38	82 338	1 428	846

a) GC strain not recognized using ELISA.

Binding Properties of the MAbs

To determine if the MAbs were directed against cell-surface exposed epitopes, hybridoma culture supernatants were incubated with intact gonococcus cells. All Mabs were able to recognize, to different degrees, their corresponding epitopes on the surface of homologous gonococcus cells (Table 2), but did not bind to *H. influenzae* cells or cells of a *N. gonorrhoeae* strain not recognized by them using ELISA.

Reactivity of the MAbs

To evaluate the reactivity of these different MAbs with *N. gonorrhoeae* and other bacterial strains, we tested a panel of microorganisms using a dot-enzyme immunoassay. None of

Table 3

Reactivity of MAbs in the Dot-Enzyme Immunoassay.

Monoclonal antibody	*Neisseria gonorrhoeae*				Other Neisseria [b]	Other bacterial species [c]
	1A (51) [d]	1B (107)	NT [a] (19)	Total (177)	(57)	(4)
NG20	30	66	8	104	0	0
NG26	31	68	10	109	0	0
NG28	42	0	5	47	0	0
NG30	1	91	9	101	0	0
NG38	2	94	8	104	0	0
Pool[e]	46	104	15	165	0	0

a) Non-typable strains.
b) N. meningitidis (39), N. cinerea (2), N. perflava/sicca (6), N. lactamica (4), N. flava (1), N. flavescens (1) and Branhamella catarrhalis (4).
c) E. coli, Staphylococcus epidermidis, Haemophilus influenzae and Streptococcus A.
d) Number of strains tested.
e) NG26, NG28 and NG38.

the MAbs reacted with *Neisseria meningitidis, N. cinerea, N. perflava, N. lactamica, N. flava, N. flavescens* nor *Branhamella catarrhalis* (Table 3). MAbs NG20 and NG26 reacted with respectively 104 and 109 strains out of 177 distributed among P1A, P1B and NT strains. MAb NG28 was positive with only 47 strains, 42 of them being P1A (82.4% of P1A strains). MAbs NG30 and NG38 reacted with 101 and 104 strains respectively. These MAbs recognized 85 and 88% of all P1B strains respectively (Table 3).

Table 4

Reactivity of a Pool of MAbs (NG26, NG28 and NG38) with GC Strains Isolated from Different Locations.

Locations	strains of GC recognized / total GC strains tested
Jamaica	3/3
Chile	2/2
Brasil	3/3
Argentina	1/1
Alberta	3/5
B.C.	2/3
Ontario	9/11
Nova Scotia	9/9
Quebec	3/3
Manitoba	5/5
Saskatchewan	1/1
unknown	124/131
Canada total	156/168
Total	165/177

Three MAbs, NG26, NG28 and NG38, were mixed together and used in a dot-enzyme immunoassay in order to recognize as many *N. gonorrhoeae* strains as possible. The strains of *N. gonorrhoeae* used for these tests were isolated from different locations in Canada and South America and represented all the different gonococcus serotypes. A total of 165/177 (93.2%) strains were picked up by the pool of MAbs (Table 4). Four of

the 12 strains missed were non-typable, 5 were P1A and 3 were P1B strains.

The limit of detection of this technique was evaluated by using serial dilutions of *N. gonorrhoeae* strain B2 (P1A) and strain T13 (P1B) incubated with MAbs NG28 or NG38. The minimum number of bacteria giving a positive reaction was between 1 and 4 x 10^4 cfu/dot, depending on the MAb.

DISCUSSION

The standard test for definitive identification of *N. gonorrhoeae* is based on culture of the organism and sugar fermentation reactions. It is well known that the gonococcus is a difficult organism to cultivate and that several environmental factors may interfere with recovery of viable organisms. In an attempt to overcome some of these problems, we have selected several MAbs recognizing surface accessible epitopes. They can be used in a dot-enzyme immunoassay which is able to detect minimal amount of *N. gonorrhoeae* antigens.

To obtain these MAbs, mice were immunized with lithium acetate extracted outer membrane. This mild procedure avoids breakage of the bacterial cells and prevents the release of internal components (15). It does not destroy the three dimensional structure of the outer membrane and allows the production of monoclonal antibodies specific to linear and conformational surface accessible epitopes. Furthermore, when these OM were used to produce hyperimmune sera in mice, 5 to 6 bands were detected by immunoblotting, indicating the immunogenicity of such preparations.

It is important to localize and identify clearly the antigens recognized by these MAbs. Western immunoblotting experiments followed by autoradiography revealed that MAbs NG30 and NG38 reacted specifically with an epitope, probably linear, on a 36 kDa molecular weight protein present on P1B strains only. MAb NG28, no matter the experimental conditions used, never reacted in Western immunoblotting, suggesting that it recognized a conformational epitope denatured by the SDS-PAGE procedure. This MAb recognized only P1A strains. Further evidence that these antibodies were directed against a protein antigenic determinant were obtained when they failed to react with OM treated with protease or proteinase K or with a preparation of LPS purified from a strain reacting with the MAb. MAbs NG20 and NG26 showed, in Western immunoblotting, a large band at the bottom of the gel, typical of LPS reaction and a band of 38 kDa molecular weight, suggesting the presence of protein-associated LPS (16). The conserved reactivity of these two MAbs with enzyme-treated OM and with purified LPS using the dot-enzyme immunoassay confirmed their specificity to LPS.

To verify the specificity of the MAbs, we used a diversified collection of *N. gonorrhoeae* strains. One hundred and seventy seven isolates were selected from a collection of strains which included different serotypes and non-typable strains. We observed that MAbs NG20 and 26, which reacted with LPS, recognized as many P1A strains and P1B strains (around 60 % respectively) and nearly half of the non-typable strains. MAb NG28 recognized the majority of P1A expressing strains (82.4%) and did not react with any P1B strains, while the P1-specific MAbs NG30 and NG38 recognized the majority

of P1B strains (85 and 87.8% respectively) and almost no P1A strains. This is consistent with the fact that these two groups of strains are mutually exclusive and that P1-specific antibodies are either P1A-specific or P1B-specific (17). All the MAbs obtained in this study were highly specific for *N. gonorrhoeae* and showed no cross-reactivity with other species of Neisseria or other unrelated bacterial species (100% specificity).

In theory, two MAbs, recognizing P1A and P1B strains respectively, should be the minimal requirement for a diagnostic test. As expected, no specific antibody reacting with all the GC strains could be found. We therefore considered the use of a mixture of MAbs with complementary reactivity. MAb NG28 was readily selected being an obvious P1A-specific antibody and MAb NG38 was included in the panel as a P1B-specific MAb. MAb NG26, LPS-specific, was also included in the pool, since it reacted with several strains missed by MAbs NG28 and NG38 and showed greater stability when conjugated to an enzyme (data not shown). When mixed together, these three MAbs (NG26, NG28 and NG38) recognized 165/177 gonococcus strains (93.2% sensitivity). Of the 12 strains missed by the mixture of three MAbs in the dot-enzyme immunoassay, 5 were from serotype Ae and Af. Because these two serotypes are seen very rarely in clinical samples, it is hoped that they will not affect the overall sensitivity of the test. Since non-identification of *N. gonorrhoeae* could cause a concern by non-treatment of infected individual, new MAbs are being characterized in order to improve the reactivity of the test against P1B and non-typable strains.

In this study, only sub-cultures of *N. gonorrhoeae* and other bacterial isolates have been tested. However, this test has the potential of being used for direct *N. gonorrhoeae*

antigen detection in clinical swabs, if the proper extraction procedure is applied. There have been reports of the direct detection of antigens in clinical specimens by Dot-enzyme immunoassay (18,19). Unlike carbohydrate-utilization tests, this test does not require pure culture, sub-culture or live organisms. Furthermore, in preliminary experiments, the MAbs have shown no cross-reaction with normal flora obtained from throat or genital swabs. The test is easy to perform and results are read by visual observation, which eliminates the need for a gamma counter, an ELISA plate reader or a fluorescent microscope.

The development of rapid, sensitive techniques such as this for the detection of *N. gonorrhoeae* infections will result in earlier treatment, reducing complications associated with this pathogen. It could also be used for routine screening to detect asymptomatic infections.

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