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MONOCLONAL ANTIBODY TO SEROTYPE 17 OF *NEISSERIA MENINGITIDIS* AND THEIR PREVALENCE IN BRAZILIAN STATES

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SUMMARY

Neisseria meningitidis are gram-negative diplococci responsible for cases of meningococcal disease all over the world. The epidemic potential of *N. meningitidis* serogroup B and C is clearly a function of their serotype antigens more than of their capsular polysaccharides. Until recently, hiperimmune sera were used to detect typing antigens on the bacteria. The advent of monoclonal antibodies (MAbs) offered the opportunity to eliminate many of the cross-reactions and have improved the accuracy and reproducibility of meningococcal serotyping. We have produced a MAb to the outer membrane protein of the already existent serotype 17 that have been detected by the use of hiperimmune rabbit sera. The prevalence of this serotype epitope is low in the Brazilian strains. By using the MAb 17 we could not decrease the percentage of nontypeable serogroup C strains. However, there were a decreasing in nontypeable strains to 13% into serogroup B strains and to 25% into the other serogroups.

KEYWORDS: *N. meningitidis* B typing.

INTRODUCTION

Neisseria meningitidis are gram-negative diplococci that colonizes the human nasopharynx and can be transmitted via droplet infection to people in close contact. Meningococcal disease (MD) is primarily a disease of early childhood, the peak incidence occurring in the first year of life. A classical high endemic area is the meningitis belt in Africa where since 1880 large outbreaks have occurred regularly. However, large epidemics have appeared in practically all areas of the world along the years [9]. Invasive strains of *Neisseria meningitidis* possess capsules consisting of high molecular weight anionic polysaccharides. Uncapsulated strains rarely cause disease [5]. Antigenic determinants on the capsular polysaccharide antigens are the basis of the classification of *Neisseria meningitidis,* into serogroup and at present there are 12 known serogroups: A, B, C, X, Y, Z, 29E, W135, H, I, K, and L[1, 9, 12], yet only serogroups A, B and C cause most of the disease.

Meaningful studies on the epidemiology of MD were not possible prior to development of methods for identification of strains within serogroups. Development of serotyping methods has enabled more precise studies to be made on the prevalence of serogroups B and C strains as a source of infection. This method has

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been used to examine strains isolated from outbreaks and to compare the serotypes of strains isolated from patients and carriers. The nature of the serotype specific determinants was investigated by FRASCH et al.[2,3]. The major outer membrane proteins of the meningococcus have been classified on the basis of their molecular weight, behavior on sodium dodecyl sufate (SDS)-polyacrylamide gels, susceptibility to proteolytic enzymes, and peptide mapping into five different classes [3,14]. The class 2 and class 3 proteins, however, appear to be functionally equivalent, and only one of them is found on any given strain. While antigenic variability has been demonstrated for outer membrane proteins of classes 1, 2, 3, and 5, only the class 1, 2 and 3 proteins are utilized to serotype meningococcal strains. Antigenic variations in the class 2 and 3 proteins in the basis for serotyping and variability in the class 1 protein are the basis for subtypes.

Until recently, hiperimmune sera were used to detect typing antigens on the bacteria; it was difficult to prepare good sera, which required extensive absorptions, and to interpret the many cross-reactions [10]. The advent of MAbs offered the opportunity to eliminate many of these problems and greatly improve the accuracy and reproducibility of meningococcal serotyping [13]. However, some of the types and subtypes are still identified using polyclonal rabbit sera due to lack of respective MAb as in the case of serotype 17. We report here production and characterization of a monoclonal antibody to the class 3 outer membrane protein of serotype 17 as well the prevalence of this serotype epitope in Brazilian strains.

MATERIALS AND METHODS

Meningococcal strains. The *N. meningitidis* serogroup B strain used for Mab production was isolated from MD in Greater São Paulo (case strain), and was initially classified as serotype and subtype: NT: P1. 14, N.337/90 strain. This strain was stored freeze-dried and in Greeve's solution at - 70°C. We also have used serotype reference strains M1080, B16B6, M986, 2996, 3006, 2396, 126E, M981, 13077, M992, M990, M978, M982, M136, S3446, H355, H44/76, 60E, 6557, 190I, 35E, BB393, 6275, 89I, 6155, 120M, 7880, 7889 [3] to characterize the new MAb. The bacteria used in this study were serosubtyped by dot-blotting [15] using MAbs kindly provided by C. E. Frasch, W. D. Zollinger and J. T. Poolman.

Outer membrane protein (OMV) preparation and purification. Outer membrane protein was prepared from the N.337/90 strain as described by FRASCH [4] with some modifications. The bacterium was grown overnight on Tryptic Soy Broth (TSB) on a gyratory shaker at 37°C and harvested by centrifugation for preparation of outer membrane vesicles. The cell pellet was resuspended in 0.1 M Tris-HCl, 0.2mM EDTA (TE) buffer, pH 8.5 (10 ml TE buffer by 1g of pellet), and sheered in a sealed Omnimixer (Sorvall) for 10 minutes with ice to avoid heating. The largely intact cells were removed by centrifugation at 10,000g and then at 12,000g for 20 minutes each. The supernatant was recovered and the OMV was pelleted at 37.800g for 45 minutes. The OMV pellet was resuspended in TE buffer and purified by gel filtration on a Sephacryl S-400 HR (Pharmacia LKB) column using TE buffer as the eluent. The OMV eluted at the void volume of column and was recovered by precipitation with 80% ethyl alcohol overnight at -20°C. Finally the OMVs were dissolved in TE buffer.

Monoclonal antibody (MAb). The methods used for production of monoclonal antibody was described previously [6]. MAb was prepared by injecting BALB/c mice i.p. three times a week for four weeks with whole cells' suspensions from *Neisseria meningitidis* N.337/90. Fusion with murine myeloma cells P3UI was performed three days after the final immunization. The hybridoma was screened by ELISA 10 to 15 days after fusion using OMVs from strain N.337/90 as antigen. A MAb was recovered which appeared to be serotype specific and was designated MAb 17. The class and subclass of the MAb were determined by ELISA using peroxidase-conjugated anti-mouse immunoglobulins as described by the manufacturer (Mouse hybridoma subtyping kit, Boehringer Mannheim).

SDS-PAGE and Immunoblotting. SDS-PAGE was performed in 12% polyacrylamide mini-gels (7 x 8cm; thickness 0.75mm Bio-Rad Laboratories) as described by LAEMMLI [8]. From 10 to 20µg OMV protein was boiled in a sample buffer containing 2-mercaptoethanol for 5 minutes. After electrophoresis, the gels were either stained with Coomassie brilliant blue R-250 or used for transfer of the separated proteins onto nitrocellulose (BA 85, Schleicher & Schuell, Dassel, FRG). The transfer was achieved by using the Semidry Transfer Cell chamber from Bio-Rad Laboratories with 25 mM Tris-192 mM glycine buffer with 20% methanol at 100 mA for 30

minutes. The immunoblots and dot-blots were probed with mouse immune sera and these antibodies were visualized by using horseradish peroxidase-IgG mouse, followed by the substrate 3-amino-9-ethyl-carbazole and hydrogen peroxidase [15].

Bactericidal assay. The microbactericidal assay was done as described by HØIBY et al., 1991[7] with minor modification. Twofold dilution of the ascites fluid of MAb 17 was tested with an inoculum of 70-80 cfu/well. A pool of normal sera obtained from a number of 4-week-old rabbits was used as a source of complement (free of bactericidal antibody to group B). After 30 min. at 37 °C, the material was plated onto Mueller-Hinton Agar plates. The bactericidal activity of MAb 17 was compared with negative and positive controls.

Screening of bacterial strains for reactivity with MAb 17. MAb 17 was used to screen 1552 *Neisseria meningitidis* strains from cases of MD isolated in Brazil during the period 1992 to 1993 by dot-blotting of whole-cell bacterial suspensions as described by WEDEGE et al. [15].

RESULTS

Monoclonal characterization. The immunoglobulin class and sub-class of the MAb of cell line F4-3C1/1A6 was determined by ELISA as IgG 2b k. This MAb was assessed for specificity in dot-blotting and western blotting experiments that employed the extracted OMV of the homologous strain and whole cell suspensions of the all prototype strains for serotypes as a target antigen. The F4-3C1/1A6 MAb only reacted with homologous strain and 6557 in dot-blotting. Strain 6557 is the prototype strain for serotype [17]. By western blotting this MAb bound to the class 3 protein of strain N.337/90 and 6557 (Fig.1). The class 3 protein was recognized with 0.25% of detergent Empigen BB added to restore the antibody binding activity to this class 3 serotype protein. After these reactions the F4-3C1/1A6 Mab was assumed to be serotype 17, and renamed MAb 17. The bactericidal activity of MAb 17 was tested by microbactericidal assay against strain N.337/90 and 6557, both strains were killed by the MAb 17.

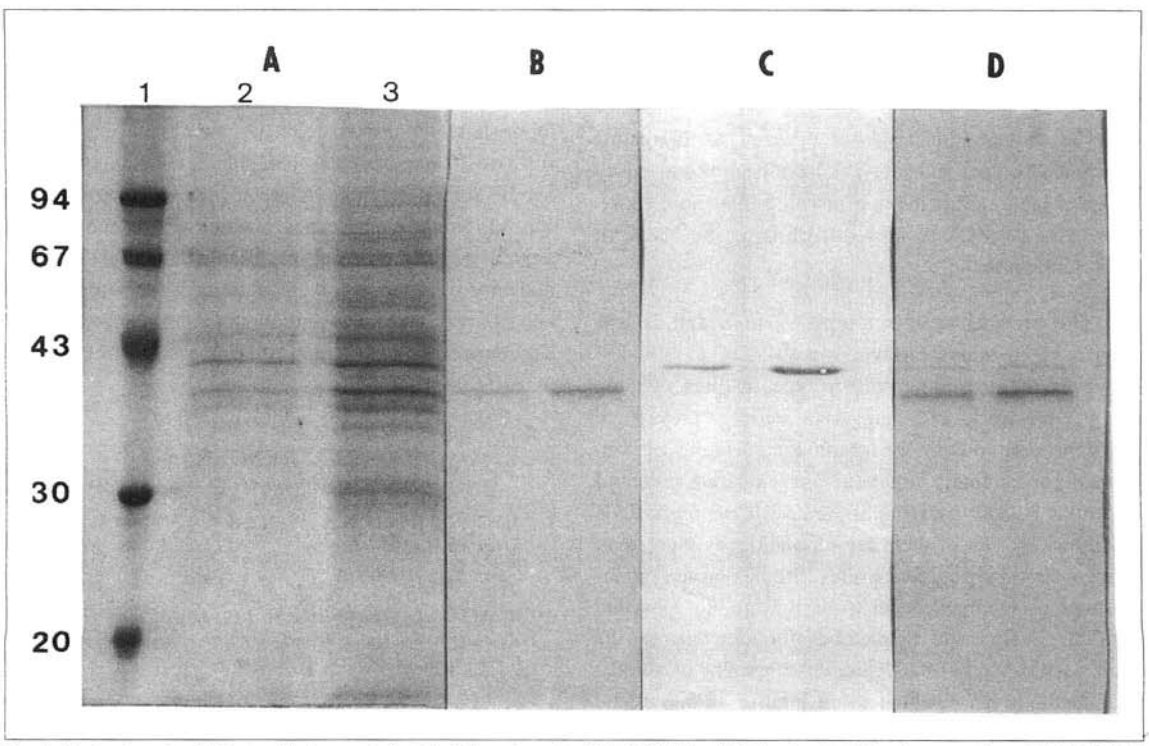

Fig. 1- Electrophoresis and Western blotting analysis of the *Neisseria meningitidis* N.337/90 and 6557 reduced whole cells suspensions. (A) SDS-PAGE of lane 1, low molecular weight (LMW), Pharmacia standard; lanes 2 and 3, N.337/90 and 6557 strains respectively. Whole cell suspensions were dissolved for electrophoresis at 100 °C for 5 minutes (A, B, C and D). Western blots of duplicate samples on the same gel without LMW standard were probed with MAb 17 (B), MAb P1.14 (MAb P1.14 used has been produced at Adolfo Lutz Institute) (C), and a mixture of MAb 17 and MAb P1.14 (D). Sizes are shown in kilodaltons.

Serotype 17 prevalence. The prevalence of the epitope 17 was estimated by screening 1552 Brazilian *Neisseria meningitidis* strains isolated from meningococcal disease during the period 1992 to 1993. Using a set of MAbs for the types 2a, 2b, 4, 8 and 15, 15% and 7% of all *Neisseria meningitidis* serogroup B and C could not be serotyped, respectively. The highest percentage of nontypeable strains was found in serogroups non B and non C, 35%. By using the MAb 17 we could not decrease the percentage of nontypeable serogroup C strains. However, there were a decreasing in nontypeable strains to 13% into serogroup B and to 25% into the other serogroups. There were no cross-reactions between MAb 17 and the other MAbs used.

DISCUSSION

The goal of this work was to characterize a MAb against the preexistent serotype 17 of *Neisseria meningitidis.* Antisera rose in rabbits using whole cell vaccines were found to have high levels of type-specific antibodies and they were used to serotype meningococcal strains by indirect hemagglutination or agar gels double diffusion (Ouchterlony technique). However, the hiperimmune rabbit sera can lead to doughty results, problems to interpret many cross-reactions and lacking in detecting strains with small amount of serotype antigens. In the last few years MAbs against different serotypes and subtypes have been produced by different laboratories making possible that more sensitive tests as ELISA and dot-blotting be used in serotyping systems.

The prevalence of serotype 17 in Brazil is low comparing to others serotypes like 4, 2b, 15 and 8[11]. However, the distribution of serotypes is quiet variable all over the world and changes in serotype prevalence are dependent on the geographic area, time period, endemic or epidemic situation. The epidemic potential of groups B, and C strains of *Neisseria meningitidis* is clearly a function of their serotype antigens more than of their capsular polysaccharides. Studies on the epidemiology of meningococcal disease were not possible prior to development of methods for identification of strains within serogroups. The development of serotyping methods has enabled more precise studies to be made on the prevalence of particular strains as a source of infection and also it is a powerful tool for tracing meningococcal epidemic strains.

The class 3 gene from the serotype 17 strain 6557 was cloned and sequenced by FRASCH et al. (unpublished data). A unique variable region was identified and a DNA probe synthesized to this region. The probe hybridized with none of the others' prototype strains, but hybridized to DNA from the strain N.337/90.

The cell line F4-3C1/1A6 and its MAb is at Institute Adolfo Lutz and it is available by request.

RESUMO

Anticorpo monoclonal contra o Sorotipo 17 de *Neisseria meningitidis* e sua prevalência em estados brasileiros

Neisseria meningitidis são diplococos Gram negativos responsáveis por casos de doença meningocócica em todo o mundo. O potencial epidêmico de *N. meningitidis* sorogrupos B e C é claramente mais uma função de seus antígenos de sorotipo que de seu polissacáride capsular. Até recentemente soros hiperimune foram usados para detectar antígenos de sorotipo em bactérias. O advento de anticorpos monoclonais ofereceu a oportunidade de eliminar muitas das reações cruzadas e têm melhorado a acuracidade e reprodutibilidade da sorotipagem de meningococo. Nós produzimos um anticorpo monoclonal contra proteína de membrana externa do sorotipo 17 que até então tem sido detectado através do uso de soro policlonal. A prevalência deste epítopo de sorotipo é baixa nas cepas brasileiras. Usando-se este anticorpo monoclonal em cepas brasileiras, não pudemos diminuir a porcentagem de cepas sorogrupo C não tipáveis, entretanto, houve uma diminuição de 13% em cepas sorogrupo B não tipáveis e 25% em cepas de outros sorogrupos.

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