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Four monoclonal antibodies against capsular polysaccharides of *Neisseria meningitidis* serogroups A, C, Y and W_{135}: Its application in identity tests

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ABSTRACT

Murine hybridoma monoclonal antibodies (MAbs) were produced against the capsular polysaccharide (CPs) of serogroups A, C, W_{135} and Y meningococci (MenA, MenC, MenW, MenY) in order to develop immunological reagents for the identification of meningococcal polysaccharides. Each serogroup-specific MAb reacted with the CPs from its homologous serogroup only and did not react with CPs from the other three serogroups. The affinity constant (Ka) of the four MAbs measured by non-competitive ELISA was 6.62×10^9, 2.76×10^9, 1.48×10^9 and 3.8×10^9 M^{-1} for MenA, MenC, MenW and MenY MAbs respectively. The application of these MAbs for identity tests was demonstrated by their abilities to correctly identify the CPs from serogroups A, C, W_{135} and Y in meningococcal CPs-based vaccines through ELISA. The MAbs obtained in this work are a very valuable set of tools for study meningococcal polysaccharides vaccines.

1. Introduction

Neisseria meningitidis is one of the major causes of bacterial meningitis and of the 13 serogroups of this bacteria, six serogroups (MenA, MenB, MenC, MenW, MenX and MenY) account for most disease worldwide [1].

There are two main types of vaccines used for protection against meningococcal infection: plain polysaccharide vaccines and protein/polysaccharide conjugate vaccines. Both are based on the CPs of the bacteria, which is a major virulence factor and is responsible for prevention of host-mediated bacterial killing [2].

Consistency of production is recognized as an important aspect of vaccine manufacture and suitable *in vitro* assays are required for quality control testing of these products [3]. For the manufacture and batch release of meningococcal polysaccharides vaccines, polysaccharides identity is a critical parameter that should be monitored. For this purpose, ^{1}H nuclear magnetic resonance (NMR) spectroscopy and/or serological assays are suitable methods for the confirmation of identity of purified polysaccharide [4]. However, NMR is a complicated and expensive technology while preparation of monospecific antisera can be a costly and time-consuming activity [5]. MAbs against CPs of *N. meningitidis* have been obtained mainly as serogrouping reagents [6,7]. This paper describes the production of four MAbs with appropriate specificities to each of the MenA, MenC, MenW and MenY polysaccharides of *N. meningitidis* and their use in an ELISA assay to evaluate the identity of each polysaccharide in meningococcal CPs-based vaccines.

2. Material and methods

2.1. Antigens

- MenAfriVac® vaccine (Serum Institute of India, Ltd): Meningococcal polysaccharide group A conjugated to tetanus toxoid (TT).
- MENVEO® vaccine (Novartis, Switzerland): Meningococcal polysaccharide groups A, C, W_{135} and Y conjugated to CRM197.
- MENCEVAX®ACWY vaccine (GlaxoSmithKline): Meningococcal polysaccharide groups A, C, W_{135} and Y.
- vax-MEN-ACW®$_{135}$ (Finlay Institute, Cuba): Meningococcal polysaccharide groups A, C and W135.
- MenA *lot PACI [2]/10*, MenC *lot PCCI [2]/10*, MenW *lot PWCI(2)/11*, MenX *lot PXCI(1)/12* and MenY *lot PYCI(1)/11*: Purified CPs used

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as internal controls provided by Reference Materials Department of Finlay Institute, Cuba.
- TT (Finlay Institute, Cuba).
- CRM197 (Sigma, USA).

2.2. Production of MAbs

For specific hybridomas against MenA polysaccharide, BALB/c mice were immunized subcutaneously (s.c) with 1 μg/dose of MenAfriVac®, with injections at 0 and 21 days. For MenC, MenW and MenY, BALB/c mice were immunized s.c with 1 μg/dose of MENVEO®, with injections at 0, 21 and 42 days. Three days before fusion a boost was given intravenously.

The fusion of spleen cells from immunized animals and SP2/0 was performed using the method of Kohler and Milstein [8]. Cells from positive wells were cloned by the limiting dilution technique and supernatants were screened by ELISA. Ascites fluids were obtained from BALB/c mice primed with pristane.

2.2.1. ELISA for hybridoma screening

Microtiter plates (Maxisorp, Nunc, Denmark) were coated overnight with either 10 μg/mL of MenA, MenC, MenW or MenY purified CPs in phosphate-buffered saline (PBS). Hybridoma supernatants from MenAfriVac® fusion procedure were added to MenA CPs-coated wells, while hybridoma supernatants from MENVEO® fusion procedure were tested simultaneously with wells coated individually with MenC, MenW or MenY CPs. Bound MAbs were detected using peroxidase-conjugated goat anti mouse immunoglobulins (anti IgG:HRP) (Sigma, USA). Normal mouse serum was used as negative control. For visualization, orthophenylenediamine (OPD) (Sigma, USA) was used as chromogen in the peroxidase reaction. Clones were considered positive when optical density (OD_{492}) was twice OD of negative control.

2.2.2. Purification of MAbs

Purification of ascites fluid was performed using HiTrap Protein G (GE Healthcare, Germany) according to the manufacturer's procedure.

Protein concentration of purified MAbs was determined using the BCA protein assay kit according to manufacturer's instructions (Pierce, USA). The purity was verified by SDS-PAGE under reducing conditions on 12.5% polyacrylamide gel [9] followed by densitometric analysis using Quantity One software (Bio-Rad).

2.3. Characterization of MAbs

MAbs isotypes were determined by using a mouse isotyping kit (Pierce, USA) according to the manufacturer's protocol.

Ka of each MAb was determined using the method described by Beatty et al. [10]. Three independent experiments were performed and results were expressed as mean of triplicate measurements.

Each MAb was evaluated against all different MenA, MenC, MenW, MenX and MenY CPs using the ELISA described in 2.2.1 to define the antibody specificity. TT and CRM197 were used as negative controls.

2.4. Evaluation of MAbs for identity tests

An indirect ELISA assay was used for identity tests.

Microtiter plates were coated with 2.5 μg/mL of MENCEVAX®ACWY, MENVEO® or vax-MEN-ACW®$_{135}$ vaccines. MAbs were added to the wells at 2 μg/ml and PBS was used as negative control. Subsequent steps were performed as described in Section 2.2.1. Each CPs were considered present in a sample if the mean OD_{492} of three replicates was twice mean OD_{492} negative control value. Three independent experiments were performed and results were expressed as mean OD_{492} of triplicate measurements.

3. Results

3.1. Production of murine MAbs against MenA, MenC, MenW and MenY CPs

Two fusion experiments were performed. From MenAfriVac® fusion procedure, twenty-six positive clones against MenA CPs were obtained. One of these clones producing a MAb of IgG2a isotype was established and designated as 7E1F7.

Eighty-eight positive clones were obtained from MENVEO® fusion procedure. Three MAbs producing IgG1 isotypes were established and named as 7E12B3, 5C11F1 and 5H10D9, recognizing MenC, MenW and MenY respectively.

Antibody purification resulted in highly purified MAbs. Densitometric analysis revealed only two bands detected around 50 kDa and 25 kDa (data not shown), representing the heavy and light chains, respectively.

3.2. MAbs specificities

All four MAbs were found to react with CPs from the homologous serogroup but did not react with related polysaccharides from the heterologous serogroups used in this study. Cross-reactivity was not detected against carrier proteins of MenAfriVac® and MENVEO® vaccines (Table 1).

3.3. MAbs affinity constant

Ka determined by non-competitive ELISA were 6.62×10^9, $2.76 \cdot 10^9$, $1.48 \cdot 10^9$ and $3.8 \cdot 10^9$ M^{-1} for MAbs 7E1F7, 7E12B3, 5C11F1 and 5H10D9 respectively.

Table 1
Antigenic specificities of antimeningococcal MAbs against capsular polysaccharides by indirect ELISA.

MAb	Mean ELISA OD_{492} against CPs from serogroup					CRM197[a]	TT[b]
	A	C	W_{135}	Y	X		
7E1F7	**2.93** ± 0.02[c]	0.06	0.06	0.065	0.075	0.072	0.06
7E12B3	0.085	**1.48** ± 0.025	0.06	0.065	0.08	0.068	0.068
5C11F1	0.065	0.06	**1.905** ± 0.021	0.06	0.07	0.07	0.065
5H10D9	0.06	0.06	0.065	**0.845** ± 0.022	0.06	0.063	0.072

Bold values indicate positive results for binding of serogroup-specific CPs.
[a] Carrier protein of MenAfriVac vaccine used as negative control.
[b] Carrier protein of MENVEO vaccine used as negative control.
[c] Mean of triplicate determinations ± SD from three independent experiments.