

Use of Poly-L-lysine Precoating in an ELISA for the Detection of Antibodies against Serogroup C *Neisseria meningitidis* Capsular Polysaccharide

✉ Rolando Ochoa Azze, Mayte Nerey Olivares, Juan C Martínez Rodríguez

DACTA. Instituto Finlay. Ave 27 No. 19805. AP 16017, CP 11600. La Lisa, Ciudad de La Habana, Cuba. Fax: (53-7) 33 6754; E-mail: ochoa@finlay.edu.cu

ABSTRACT

Precoating with poly-L-lysine was analyzed in an ELISA used for the quantitative determination of antibodies against serogroup C *Neisseria meningitidis* capsular polysaccharide. Different coatings were analyzed: purified capsular polysaccharide of serogroup C *N. meningitidis* did not attach well to polystyrene, but precoating with poly-L-lysine and the use of methylated human serum albumin attached to polysaccharide had a good performance. Cross reactivity to poly-L-lysine was not detected in 48 adults immunized with a divalent (serogroups B and C) meningococcal vaccine (VA-MENGOC-BC®). Seroconversion and seroresponse were detected in 89.58 and 97.92% of the vaccinated individuals, respectively.

Keywords: ELISA for anti-polysaccharide C antibodies, poly-L-lysine, poly-L-lysine precoating, serogroup C *Neisseria meningitidis*

Biotechnologia Aplicada 1999;16:173-175

RESUMEN

Se evaluó el prerecubrimiento con poli-L-lisina en un ELISA para la cuantificación de anticuerpos contra el polisacárido capsular de *Neisseria meningitidis* serogrupo C. Se analizaron diferentes recubrimientos: el polisacárido C no se unió bien al poliestireno, pero se alcanzaron buenos resultados recubriendo previamente con poli-L-lisina, así como con seroalbúmina humana metilada unida al polisacárido C. No se detectó reactividad cruzada a la poli-L-lisina en 48 adultos inmunizados con una vacuna anti-meningocócica bivalente contra los serogrupos B y C (VA-MENGOC-BC®). Se detectó seroconversión en 89,58% de los vacunados y serorrespondió 97,92%.

Palabras claves: ELISA para anticuerpos anti-polisacárido C, *Neisseria meningitidis* serogrupo C, poli-L-lisina, prerecubrimiento con poli-L-lisina

Introduction

Although ELISA procedure has been commonly used for measuring antibodies to protein antigens, its application for polysaccharides has not been successful. Negatively charged polysaccharides do not attach easily to polystyrene in ELISA [1-3]. In order to solve this problem, various methods have been developed.

The uses of capture antibodies [3] are limited because of the extreme difficulty to produce specific immunological reagents. Covalent binding to proteins [1] is tedious and introduces the risk of altering the immunological reactivity. The avidin-biotin method has been used [3-5], but careful methods must be employed to avoid conformational alteration. Methylated human serum albumin (mHSA) has been successfully used to coat with serogroup A and C capsular polysaccharides of *Neisseria meningitidis* [6, 7], but it is not commercially available and it is difficult to produce. Precoating with poly-L-lysine (PLL) [8-10] has been criticized because it generates a low signal to noise ratio and non-specifically reacts with some sera [3, 5].

We examined the performance of an ELISA using precoating with PLL to measure the antibody response to the serogroup C polysaccharide in subjects vaccinated with a divalent (serogroups B and C) meningococcal vaccine.

Materials and Methods

Meningococcal polysaccharide

The capsular polysaccharide (CPS) antigen of serogroup C *N. meningitidis* was provided by the

"Finlay" Institute, Havana, Cuba. The polysaccharide contained less than 1% of protein and nucleic acids.

Serum specimens

Forty-eight volunteer adults over 40 years old were immunized with VA-MENGOC-BC® (divalent [serogroups B and C] meningococcal vaccine). The vaccination scheme consisted in two doses of 0.5 mL, with an interval of 8 weeks. Known vaccinations with VA-MENGOC-BC® or other meningococcal vaccine in the last 10 years, and antecedents of meningitis were exclusion criteria. Venepuncture was performed immediately prior to the first dose (T0) and four weeks after the second dose (T2). Sera were stored at -20 °C until simultaneous processing.

ELISA method for anti-polysaccharide antibodies

High-binding ELISA plates (Costar, Cambridge, Ma) were precoated by incubation with 100 µL of PLL per well (3 µg/mL) (Sigma Chemical Co, St. Louis, MO) in 0.15 M phosphate buffered saline (PBS), pH 7.4 for 30 min at 20-25 °C. After the plates were washed three times with 300 µL of PBS per well, 100 µL of serogroup C *N. meningitidis* CPS (2.5 µg/mL) in PBS was added to each well. The plates were incubated overnight at 2-8 °C and then washed three times with PBS containing 0.05% Tween 20 (PBS-T). The standard curve was constructed by performing six two-fold serial dilutions of the "Finlay" standard serum

1. Gray BM. ELISA methodology for polysaccharide antigens: protein coupling of polysaccharides for adsorption to plastic tubes. *J Immunol Methods* 1979;28:187-92.

2. Barra A, Schulz D, Aucouturier P, Preud'homme JL. Measurement of anti-*Haemophilus influenzae* type b capsular polysaccharide antibodies by ELISA. *J Immunol Methods* 1988;115:111-7.

3. Díaz Romero J, Outschoorn IM. Detection of antibodies to *Neisseria meningitidis* group B capsular polysaccharide by a liquid-phase ELISA. In: Roth J, Rutishauser U, Troy II FA, editors. *Polysialic Acid*. Switzerland: Birkhäuser Verlag Basel; 1993. p.39-48.

4. Sutton A, Van WF, Karpas AB, Kathryn ES, Schneerson R. An avidin-biotin based ELISA for quantitation of antibody to bacterial polysaccharides. *J Immunol Methods* 1985;82:215-24.

5. Díaz Romero J, Outschoorn IM. Selective biotinylation of *Neisseria meningitidis* group B capsular polysaccharides: application to an improved ELISA for the detection of specific antibodies. *J Immunol Methods* 1993;160:35-40.

6. Gheesling LL, Carlone GM, Pais LB, Holder PF, Maslanka SE, Plykaytis BD, et al. Multicenter comparison of *Neisseria meningitidis* serogroup C anti-capsular polysaccharide antibody levels measured by a standardized enzyme-linked immunosorbent assay. *J Clin Microbiol* 1994; 32:1475-82.

(10 000 U/mL) (starting dilution 1:100) in PBS-T containing 3% skim milk (PBS-TM) (Merck, Germany). PBS-TM was used as zero-standard. The test sera and the control serum were diluted 1:100 in PBS-TM. Of each solution, 100 µL were added in duplicate to the wells for 1 h at 37 °C. After washing four times with 300 µL of PBS-T, 100 µL of conjugate solution (goat anti-human IgG:alkaline phosphatase) (Sigma, St. Louis, MO) in PBS-TM were added to each well and the plates were incubated for 1 h at 37 °C. The plates were washed again, and 100 µL of p-nitrophenylphosphate (1 mg/mL) (Sigma, St. Louis, MO) in 0.92 M diethanolamine buffer pH 9.8 were added to each well. The plates were left at 20-25 °C for 30 min and then the absorbances were determined at 405 nm (Anthos reader 2001, Labtec Instruments, Germany) [9]. Validation, quantitative determination and print out were performed using the ELISA software package from the Center for Disease Control (CDC), Atlanta, USA [11].

Detection limit was calculated after the blank reagent PBS-TM was tested 80 times in three performances. The mean plus twice the standard deviation was taken as an estimate of the detection limit [12]. The absorbance values were homogenized and transformed to U/mL by the ELISA software package from the CDC [11] using a reference curve. Inter-assay average was calculated.

Half the value of the detection limit was assigned to samples with concentrations below the detection limit.

ELISA method used to detect non-specific reaction against PLL

Wells of columns 1-4 of high-binding ELISA plates were sequentially coated with PLL and serogroup C *N. meningitidis* CPS as it was described above. Columns 5-8 were only coated with PLL for 30 min at 20-25 °C, and after washing, wells were soaked in PBS. PBS was also added to wells of the remaining columns, i.e. wells without coating. After incubation overnight at 2-8 °C, the plates were washed three times with PBS-T. The serum samples collected prior to the first dose, which were above the detection limit for anti-polysaccharide antibodies, were diluted 1:100 in PBS-TM. Of each sample, 100 µL were added in duplicate to coated and non-coated wells, and the plates were incubated for 1 h at 37 °C. The next steps were performed as previously described.

ELISA method used to analyze different coatings

Sequential coating with PLL and CPS was used as described above in columns 1-4 of a plate. Columns 5-8 were coated with an equal mixture of 5 µg of CPS/mL and mHSA/CPS (gently provided by CDC) [6]. Columns 9-12 were only coated with CPS (10 µg/mL). The next steps were as described. The standard curve was used to analyze the coatings.

Statistical analysis

Statistical analysis was carried out using appropriate tests in the Statgraphic software [13] on an IBM compatible computer.

Linear regression analysis between standard curves was used to analyze different coatings with respect to

mHSA/CPS. Analysis of variance for checking the linear model and the Cochran's C test for homogeneity of variances were performed.

Geometric means and 95% confidence intervals were calculated for anti-polysaccharide C antibody distribution in T0 and T2. Comparison of means was carried out by Student's t test for matched samples after Chi-square test for normality was performed.

The T2/T0 relation was calculated for each pair of samples. The relation ≥ 4 was defined as seroconversion and ≥ 2 as seroresponse [10].

Results and Discussion

Meningococci are divided into a number of serogroups on the basis of structural differences in the CPS. The serogroups B, C, Y and W-135 have sialic acid in their CPS. Sialic acid-containing polysaccharides confer resistance to host complement-mediated attack mechanisms. The purified CPS vaccine against serogroup C was among the first chemically pure bacterial vaccines [14, 15]. This vaccine is immunogenic, safe and effective.

The antibody response has shown to correlate well with the bactericidal assay [6, 10, 16, 17], but the measurement by ELISA of antibodies to purified cell surface carbohydrates is often hindered by the failure of lipid-free or protein-free polysaccharides to efficiently and reproducibly bind to a solid phase [1, 2, 5]. On the other hand, the acidic polysaccharides attach well to the basic PLL, so we used it.

Precoating with PLL, which has reactive epsilon-amino groups, resulted in high antibody binding with low background. The standard curve for PLL+CPS and mHSA/CPS was similar. The coating with CPS showed the smallest sensitivity (Table 1). Sensitivity is defined for quantitative immunoassays as the ability to detect small differences in concentration, it is, the ratio of the change in response of the method to the change in concentration of the analyte, or the slope of the analytical calibration curve [12].

The detection limit is the concentration distinguishable from the zero-standard. It was 367 U/mL. Only seven serum samples collected in T0 were above the detection limit. Cross reactivity to PLL was not detected in any sample (Figure). On the other hand, PLL showed a blocking role on the polystyrene surface.

Table 1. Analysis of different coatings used in an ELISA for the detection of antibodies against serogroup C *N. meningitidis* capsular polysaccharide.

Points of the curve	mHSA/CPS (mean OD)	PLL + CPS (mean OD)	CPS (mean OD)
1	0.187	0.238	0.116
2	0.312	0.426	0.196
3	0.529	0.690	0.293
4	0.943	1.077	0.551
5	1.392	1.457	0.909
6	1.787	1.814	1.315
Slope	-	0.96	0.73
Intercept	-	0.12	-0.06
Coefficient of determination	-	0.99	0.98
Analysis of variance	-	p = 0	p = 0
Cochran's C test	p = 0.99	p = 0.96	p = 1

mHSA/CPS: coating with an equal mixture of serogroup C *N. meningitidis* capsular polysaccharide and methylated human serum albumin.

PLL + CPS: sequential coating with poly-L-lysine and serogroup C *N. meningitidis* capsular polysaccharide.

CPS: coating only with serogroup C *N. meningitidis* capsular polysaccharide.

OD: optical density.

7. Carlone GM, Frasch CE, Siber GR, Quaalert S, Gheesling LL, Turner SH, et al. Multicenter comparison of levels of antibody to the *Neisseria meningitidis* group A capsular polysaccharide measured by using an enzyme-linked immunosorbent assay. *J Clin Microbiol* 1992;30:154-9.

8. Leinonen M, Frasch CE. Class-specific antibody response to group B *Neisseria meningitidis* capsular polysaccharide: use of polylysine precoating in an enzyme-linked immunosorbent assay. *Infect Immun* 1982;38:1203-7.

9. Nerrey M, Ochoa R, Martinez JC, Licea T, Ferriol X, Garcia AM, et al. ELISA para la cuantificación de IgG humana anti-polisacárido C de *Neisseria meningitidis*. En: Gavilondo JV, Ayala M, Acevedo B, Bello M, editores. *Biocología Moderna '97. Avances en Biotecnología Moderna*; 1997 Dic 1-6; La Habana, Cuba. La Habana: Elfos Scientiae; 1997. p.30.

10. Echeverry ML, Malbert JA, Galeano LA, Sotolongo FT, Galguera MA, Montoya CM, et al. Respuesta inmune humoral al polisacárido capsular de *Neisseria meningitidis* serogrupo C en un ensayo de vacunación antimeningocócica BC en Antioquia, Colombia. *Bol Oficina Sanit Panam* 1995;118:295-301.

11. Program ELISA [computer program]. MS DOS version. Atlanta, Ga: Center for Disease Control; 1993.

12. Chaloner-Larsson G, Anderson R, Egon A. A WHO guide to good manufacturing practice (GMP) requirements. Part 2: Validation. Geneva: WHO; 1997. p.65-95.

13. Statgraphics® plus for Windows™ [computer program]. Version 1.0. Statistical Graphics Corp. USA; 1994.

14. Frasch CE. Meningococcal vaccines: past, present and future. In: Cartwright K, editor. *Meningococcal Disease*. Gloucester: John Wiley & Sons Ltd; 1995. p.246-83.

15. Ala'Aldeen DAA. Vaccine against *Neisseria meningitidis*: Past, present and future. *Biocología Aplicada* 1996;13:1-7.

Specific reaction to CPS before vaccination could be explained by a long-lasting protective immunity elicited by VA-MENGOC-BC®. Cross reactivity with *Escherichia coli* K92 CPS is less probable because strains of this pathogen have been infrequently found in healthy individuals. Cross reactivity with other CPS has not been demonstrated yet, and serogroup C meningococci are rarely found among healthy individuals [13, 18]. Certainly, vaccination with a serogroup C meningococcal vaccine can not be disregarded, therefore, specific reaction to CPS should be carefully studied to reach a conclusion.

Even though certain sera could react non-specifically against PLL, this ELISA is useful to evaluate

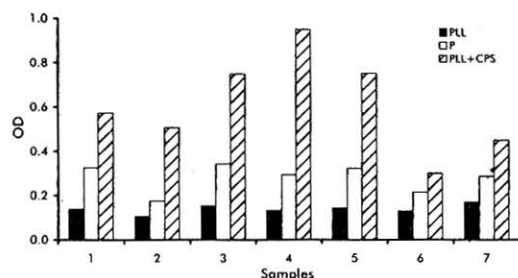


Figure. Reactivity against serogroup C *N. meningitidis* capsular polysaccharide (PLL+CPS), poly-L-lysine (PLL), and binding to polystyrene (P) of specimens with a high signal before vaccination.

Received in September, 1998. Accepted for publication in October, 1998.

the immune response against the serogroup C polysaccharide-based vaccine, and probably for other acidic polysaccharide-based vaccines. The mean of samples collected after vaccination was significantly larger than the mean of samples collected before vaccination (Table 2); and seroconversion ($T2/T0 \geq 4$) was detected in 43 vaccinated individuals (89.58%), including those with initial reactivity to CPS. Seroreponse ($T2/T0 \geq 2$) was found in 47 individuals (97.92%). Other investigations showing a high seroconversion and seroreponse [10, 15] support this method. Nevertheless, the background reactivity to PLL of each sample could be subtracted if necessary [19].

In conclusion, our results support that precoating with PLL is a suitable method for the ELISA used to detect antibodies against serogroup C *N. meningitidis* CPS.

Table 2. Results obtained in serum samples tested for anti-polysaccharide C antibodies before and after vaccination.

	T0	T2
N	48	48
Geometric mean	248	3656*
95% confidence intervals	200 - 307	2763 - 4839

T0: serum samples collected before vaccination.

T2: serum samples collected four weeks after the second dose.

*Student's t test $p = 1.4 \times 10^{-21}$.

16. Fernández JA, Malberty JA, Sotolongo F, Bacallao J, Camaraza MA, Nerey MC, et al. Cinética de la respuesta de anticuerpos bactericidas y de la IgG específica en individuos vacunados con VA-MENGOC-BC®. Rev Cubana Hematol Immunol Hemoter 1997;13:38-45.

17. Mitchell LA, Ochnio JJ, Glover C, Lee AY, Ho MKL, Bell A. Analysis of meningococcal serogroup C-specific antibody levels in British Columbia children and adolescents. J Infect Dis 1996;173:1009-13.

18. Devi SJN, Robbins JB, Schneerson R. Antibodies to poly [(2-8)- α -N-acetylneuraminic acid] and poly [(2-9)- α -N-acetylneuraminic acid] are elicited by immunization of mice with *Escherichia coli* K92 conjugates: potential vaccines for groups B and C meningococci and *E. coli* K1. Proc Natl Acad Sci USA 1991;88:7175-9.

19. Andersen J, Berthelsen L, Lind I. Measurement of antibodies against meningococcal capsular polysaccharide B and C in enzyme-linked immunosorbent assay towards an improved surveillance of meningococcal disease. Clin Diag Lab Immunol 1997;4:45-51.



Biodiversidad y biotecnología de la caña de azúcar

Ariel D. Arencibia
María T. Cornide

Elfos
SCIENTIAE

La caña de azúcar es un cultivo básico en muchos países tropicales. Su importancia económica ha motivado el interés por aplicar en su cultivo las más novedosas técnicas de la biotecnología moderna y la biología molecular.

Este libro, primero editado por Elfos Scientiae en el campo de la biotecnología vegetal, será sin dudas útil a los investigadores y mejoradores dedicados a la obtención de variedades de caña de azúcar, tanto por métodos tradicionales como biotecnológicos. Los puntos de vista que se discuten pudieran ser útiles a especialistas dedicados al mejoramiento de otras especies con características similares a las de este

Entre otros temas...

- Biodiversidad disponible
- Los análisis del polimorfismo del ADN
- Determinación de fuentes genéticas con fines de mejoramiento
- Localización de genes para su uso en ingeniería genética
- Selección de nuevas variedades mediante marcadores moleculares
- Transgénesis y sus aplicaciones comerciales
- Estudios fisiológicos y posibilidades futuras de biofertilización

Precio: \$20.00 USD

ISBN 959-235-013-2

Envíe su solicitud a:

Elfos Scientiae
Apartado 6072,
La Habana 6, Cuba.
Tel: (53-7) 33 1917
(53-7) 21 8164
Fax: (53-7) 33 1917
(53-7) 33 6008
E-mail: elfos@cigb.edu.cu