

Opsonization and transformation: effects of anticapsular sera on the DNA transfer in *Neisseria meningitidis*

Opsonização e transformação: efeitos do soro anticapsular sobre a transferência de DNA em *Neisseria meningitidis*

RIALA6/1511

Gisele Cristiane Gentile CURY, Rafaella Fabiana Carneiro PEREIRA, Luciana Maria de HOLLANDA, Marcelo LANCELOTTI*

*Corresponding Address: Department of Biochemistry, Institute of Biology, State University of Campinas – UNICAMP, CP6109, 13083-970, Campinas, SP, Brazil. Tel: +55 19 3521 6150, Fax: +55 19 3521 61 29. E-mail: mlancell@unicamp.br
Recebido: 30.08.2011 – Aceito para publicação: 30.09.2012

RESUMO

O processo de comutação capsular indica a ação de anticorpos capsulares específicos na adaptação de linhagens de meningococo. Foram empregados diferentes anticorpos para verificar o efeito da opsonização sobre a transformação de *Neisseria meningitidis* dos sorogrupos C e W135. Essas análises mostraram o bloqueio da transformação pela ação de anticorpos capsulares específicos ao meningococo, demonstrando assim o efeito bloqueador da ação desses componentes sobre o processo de transformação de *N. meningitidis*. Tal efeito pode estar ligado com o processo de comutação capsular e abre novos campos para exploração científica.

Palavras-chave. *Neisseria meningitidis*, transformação, opsonização, cápsula.

ABSTRACT

The capsular switching process indicates the action of specific capsular antibodies on the meningococcal strains adaptation. Different antibodies were employed for assessing the effect of opsonization on the transformation of *Neisseria meningitidis* serogroups C and W135. These analyses showed the blocking action of the specific capsular antibodies on the meningococcal transformation capacity. Thus, the blocking effect of these antibodies on *N. meningitidis* transformation process was demonstrated. This effect could be involved in the capsular switching process and the found data might open new subjects for scientific exploration.

Keywords. *Neisseria meningitidis*, transformation, opsonization, capsule.

INTRODUCTION

Neisseria meningitidis or meningococci (Mc) is a Gram negative bacterium that may cause meningitis and septicemia outbreaks in several regions of the world. As *Haemophilus influenzae* and *Streptococcus pneumoniae*, these natural competent bacteria for transformation by DNA, colonize the upper respiratory tractus in human. Outer membrane proteins are involved in DNA uptake¹. These proteins recognize and transfer to cytoplasm DNA fragments containing specific uptake sequences (US). In *H.influenzae* the US is composed of 9bp (5'AAGTGCGGT3') while in *Neisseria* the US is composed of 10bp first recognized in *N.gonorrhoeae* (5'GCCGTCTGAA3'). Furthermore, outer membranes vesicles (bleb's) are also described as carrier of DNA in *Neisseria*².

Transformation was involved in capsule switching in *N. meningitidis*. Capsular switching events are expected to occur continuously and can be selected by immune response against a particular capsular polysaccharide^{3,4}. However, the interference of immune response with transformation efficacy has not yet evaluated. Specific capsular antibodies are expected to bind to the bacterial surface and hence the interference in DNA recognition and uptake. In this work we aimed to explore the action of the opsonization process on DNA transformation in *N. meningitidis*, using different serogroup specific antibodies.

The strains C2135 its derived KZ1 (serogroup C strain harbouring *pilC1:lacZ:aphA3'*) and W135atcc and its derived W03.01. Bacteria were grown at 37°C under 5% CO₂ on chocolate agar. When needed, kanamycin or erythromycin were added to the medium at 100 µg/mL and 2 µg/mL, respectively. The disrupted *synG* mutant was designed for inactivated *synG* gene responsible for the synthesis of the W135 capsular polysaccharide⁵.

Reciprocal transformations assays of these two strains were performed in triplicate assays. Bacteria were first incubated with a rabbit serum directed against serogroup C or serogroup W135 capsule. After incubation for 1h at 37 °C, 100 µL of the opsonized bacterial suspension was mixed with 5 µg of genome DNA from KZ1 or W03.01 strains. After 5 hours of incubation at 37 °C 5% CO₂, transformants were selected on chocolate plates supplemented with appropriate antibiotics. All assays were performed without any

source of complement. All antibodies did not show any bactericidal effects under these experimental conditions.

Genomic DNA from strain KZ1 was used to transform the strain C2135. The frequency transformation dropped from 2.5×10^{-6} to 5×10^{-7} and 5×10^{-8} after opsonization with anti-capsular antibodies. No such a reduction was observed when transformation was performed in the presence of anti-pilin antibodies. Both types of antibodies similarly recognized the strain C2135 (data not shown). Similar results were obtained when the strain W135atcc was transformed by DNA from strain W03.01 in the presence or absence of anti capsular W135 antibodies (data not showed).

We next tested the effect of anti-capsule serum on the transformation in a co-culture experiment with KZ1 and W03.01 strains at equivalent concentrations of 1.10^8 CFU/mL in BHI broth. We scored the transformants exhibiting resistance to both kanamycin and erythromycin in presence of serogroup C or W135 anti-capsule sera at 1/50 and 1/100 dilutions. Our results showed a drastic reduction in the frequency of transformation in the presence of either of these sera when compared to transformation experiment in the absence of sera (Figure 1). Also, our data indicate the presence of the capsule does not inhibit the transformation and the opsonization process was able to inhibit the transformation. Indeed, the mechanisms of DNA uptake in *Neisseria* genus are known in *N. gonorrhoeae*, a non-capsulated bacteria whereas many proteins had been identified as responsible for the uptake system¹. The role of capsule specific antibodies may be relevant after vaccination to limit DNA transfer in strains tagged by the vaccination.

Table 1. Strains of *Neisseria meningitidis* used in this work

Strain	Description	Reference
C2135	<i>Neisseria meningitidis</i> serogroup C, BIOMERIEUX is derived from KZ1	INCQS - FIOCRUZ
KZ1	Derived from LNP8013 clone 12 <i>pilC1:lacZ:aph3'</i> transcriptional fusion	Taha et al. ⁶
W135 atcc	<i>Neisseria meningitidis</i> serogroup W135, ATCC35559	INCQS - FIOCRUZ
W03.01	Derived from W135 atcc containing the <i>ermAM</i> cassette into <i>BclI</i> site of <i>synG</i>	This work

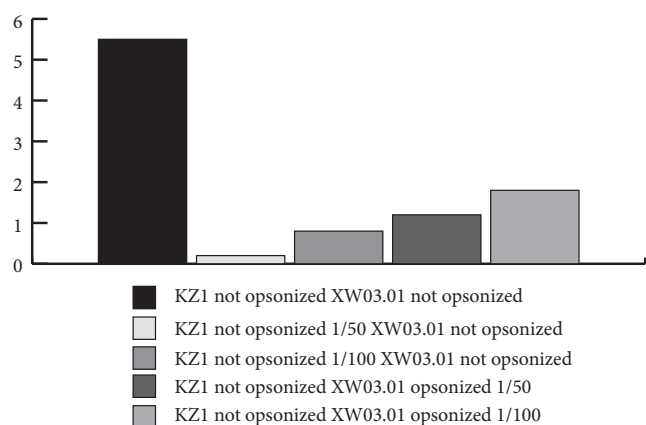


Figure 1. Transformation Frequency after co-culture assays between KZ1 and W03.01. This histogram show the differences into transformation frequency submitted to opsonization process of the meningococcal strains. The strains were opsonized with rabbit antibodies specific to the serotype C and W135 capsular polysaccharide.

ACKNOWLEDGEMENTS

Thanks for Dr. Taha and Dr. Alonso from Pasteur Institut for gift the rabbit antisera. The LABIOTEC was supported by FAPESP (number 2008/56777-5) and CNPq (number 575313/2008-0). LMH received grants to CAPES funding by Nanobiotechnology Program.

REFERENCES

1. Dubnau D. DNA uptake in bacteria. *An Rev Microbiol*. 1999;53:217-44.
2. Tortosa P, Dubnau D. Competence for transformation: a matter of taste. *Curr Opin Microbiol*. 1999;2(6):588-92.
3. Perez-Trallero E, Vicente D, Montes M, Cisterna R. Positive effect of meningococcal C vaccination on serogroup replacement in *Neisseria meningitidis*. *Lancet*. 2002;21;360(9337):953.
4. Taha MK, Parent Du Chatelet I, Schlumberger M, Sanou I, Djibo S, de Chabaliere F, et al. *Neisseria meningitidis* serogroups W135 and A were equally prevalent among meningitis cases occurring at the end of the 2001 epidemics in Burkina Faso and Niger. *J Clin Microbiol*. 2002;40(3):1083-4.
5. Hollanda LM, Cury GC, Pereira RF, Ferreira GA, Sousa A, Sousa EM, et al. Effect of mesoporous silica under *Neisseria meningitidis* transformation process: environmental effects under meningococci transformation. *J Nanobiotechnol*. 2011;9:28.