Mucosal approaches in *Neisseria* Vaccinology

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Introduction

Meningococcal B strains accounts for some 72% and 28% of meningococcal diseases in infants and toddlers of Europe and in the USA, respectively (1,2). Nevertheless, meningococcal diseases are rare in Cuba owing to the wide spread program on anti-meningococcal vaccination in the country (3).

Finlay Institute is one of the pioneering organizations in *Neisseria* Vaccinology mainly by its contribution to *N. meningitidis* serogroup B outer membrane-based bivalent vaccine, VA-MENGOC-BC® (4). This was applied, intramuscularly, in more than 60 million doses. Only 10.7 millions of them correspond to Cuban young adults, children, and infants. However, most dangerous or commensally *Neisseria* strains enter and establish in the mucosa, where the secretory (S) IgA is the main specific guardian and is mainly induced by mucosal routes (5). In addition, colonization of young children by *N. lactamica* has been implicated in naturally-acquired meningococcal immunity. Nonetheless, more attention was dedicated to systemic response against *Neisseria* diseases. Contrary, less attention was dedicated to mucosal immune response. In addition, few mucosal vaccines exist predominantly due to the absent of safe mucosal adjuvants.

We develop a Finlay Adjuvant (AF) platform based in outer membrane vesicles (Proteoliposome, PL) and its derivate Cochleate (Co). AFPL1 derived from serogroup B *N. meningitidis* is a potent Th1/CTL driving parenteral adjuvant. AFCo1 is a potent mucosal adjuvant (6,7). Therefore, we sought the possible mucosal cross recognition between *N. meningitidis* serogroups and *Neisseria* species and explore a concurrent mucosal and parenteral immunization strategy (SinTimVaS) in order to develop suitable mucosal vaccines.

Mucosal cross recognition between *N. meningitidis* serogroups and *Neisseria* species

As human mucosa is involved in *Neisseria* colonization and it is mainly specifically protected by SIgA, we evaluated the recognition of SIgA of different *Neisseria*. First, we selected human parotid saliva from subjects with anti PL IgA response in ELISA test. Sera from the same subjects were also obtained. Second, we evaluate in western blotting membrane antigens from different *N. meningitidis* serogroups B (Cu385/83), A (ATCC 13077), C (C11), Y (6304), and W_{135} (S4383). All serogroups were recognized by saliva IgA with similar pattern than serum IgG. Third, recognition of different *Neisseria* species was conducted. These SIgA and IgG cross recognition responses were also observed between pathogenic (*N. meningitidis* serogroup B, *N. gonorrhoeae*) and non-pathogenic strains (*N. flava*, *N. lactamica*). These observations extend the knowledge of some cross recognition at IgG level in sera to mucosal environments where the same proteins patterns were identified.

Parenteral meningococcal vaccines increased saliva IgA in *Neisseria* carriers

Human parenteral vaccines do not normally induce mucosal IgA which is confirmed in mice immunized with intramuscular doses of VA-MENGOC-BC® Contrary, nasal AFCo1 in mice (8) and nasal experimental *N. lactamica* colonization in human induces systemic (IgG) and mucosal (IgA) specific responses (9). Consequently, we sought the influence of parenteral meningococcal boost over mucosal response during *Neisseria* carriage. Only those carrying *Neisseria* boost saliva anti PL SIgA. This result could explain the induction of specific IgA observed in some subjects after meningococcal parenteral immunization.

Possible influence of Meningococcal vaccination over *N. gonorrhoeae* incidence in Cuba

As there are serum and mucosal cross recognition between *Neisseria* species, long-lasting persistence of specific IgG induced by VA-MENGOC-BC®, and the consensus that transudation of IgG in addition to SIgA protect the mucosa we sought the possible influence of meningococcal vaccination over *N. gonorrhoeae* incidence in Cuba.

First, the statistic report of meningococcal, Gonorrhea and other sexual-transmitted diseases incidence of Cuban Ministry of Heath was analyzed. The maximum incidence of meningococcal disease occurred in 1983. However, *N.*

gonorrhoeae have two picks one in 1989 and the other in 1995, but from 1982 to 1999 the incidence were over 20000 cases by year. Nevertheless, after 1995 *N. gonorrhoeae* declined in parallel *N. meningitidis*, but with around 12 years of retard when sexual activity began (Figure 1). In addition, Syphilis and Condiloma behavior not change during this time (data not shown).

Second, the meningococcal epidemiological situation and the meningococcal serogroups A+C campaign during 4 months of 1979 were reviewed. In this year, *N. meningitidis* serogroup C was the main isolated from cases with 50.9%, followed by 34,3% of serogroup B and 1.4% of serogroups A and Y. The vaccination covered the 78,2% of people less than 20 years old (Tab. 1). After this intervention the serogroups C and A practically disappear and increased dramatically the serogroup B. This behavior was also observed at carrier isolated where serogroup B predominated. This vaccination had not a direct impact over *N. gonorrhoeae* incidence (Figure 1).

Third, the meningococcal serogroups BC vaccination campaign (VA-MENGOC-BC®) from December 1988 to December 1990 was analyzed.This was performed from December 1988 to December 1990. The vaccination covered 75.38% people from 3 months to 19th years old. This covered the 86.2; 75 and 65,6% from 3 months to 5 years, 6 to 14 years, and 15 to 19 years old, respectively. Consequently, this includes more than 66% of people during sexual activity ages in a short period of time (Tab. 1).

An immediately drop in the prevalence of *N. gonorrhoeae* incidence was observed (Figure 1). If we accept that during epidemic periods also the carrier prevalence is increased and that carriage induces SIgA, this intramuscular vaccination campaigns could increased in addition to serum specific IgG, also SIgA. Mucosal IgA are more cross reactive (innate like) than serum IgG (5), but also we demonstrated specific cross recognition between both *N. meningitidis* and *N. gonorrhoeae* species. Both, cross recognition and cross reactive serum IgG and SIgA could be involved in this Gonorrhea drop.

Fourth, the VA-MENGOC-BC® introduction in the Cuban (national immunization program, NIP) since 1991 was analyzed. This includes two doses at 3 and 5 months of age whit coverage of around 99% of infants. In total campaign plus PIH our population receive around 10,7 million doses. This vaccine reduce the meningococcal morbidity over 95% and its mortality over 98%.

As this vaccine since 1991 is applied to infants its possible influence over sexual transmitted Gonorrhea take at least 12 years. Nevertheless, long-lasting specific IgG at least for 12 years after vaccination was observed (10,11). We observed higher induction of cellular immune response in serum IgG negative vaccinated people than in IgG positive subjects. The presence of carriers influences but not eliminated by vaccination could challenge human add complexity to these mucoca-parenteral interactions.

Fifth, other factors that could influence the *N. gonorrhoeae* incidence were also analyzed. Between them are: increased of special programs related with sexual transmitted diseases, since 2000 and condom accessibility that occur around 2003. These measures occur after the *N. gonorrhoeae* decrease. Particular require the special period live in Cuba during the beginning of 90s where diagnostic difficulties between other occur. This could explain the increased in Gonorrhea incidence during 1994 with a maximum in 1995. This is important to note that neither specific vaccine nor other specific anti Gonorrhea measured was introduced.

Sixth, as mucosal cross recognition between *Neisseria* species was found as describe above we hypothesized that nasal AFCo1-derived from serogroup B *N. meningitidis* could also induces immune recognition of *N. gonorrhoeae*. Mice were immunized with 3 nasal doses of AFCo1 and antibodies and T cell response were measured. For more results see Cuello M, et al, in this edition (12).

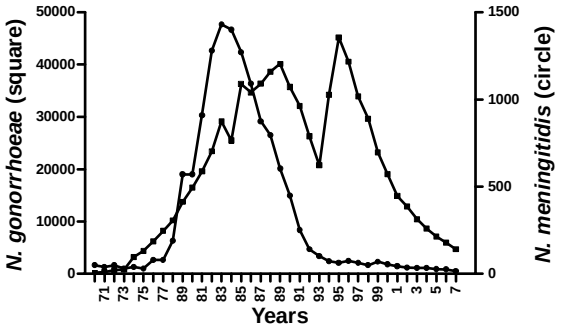
Seventh, last but not least, we develop a SinTimVaS using concurrent doses of nasal AFCo1 and parenteral AFPL1 and

Tabla 1. Distribution of ages and coverage of Meningococcal vaccination campaigns in Cuba, (m, month and y, year)

Vaccination campaign	Year	Age	Population	Vaccinated	Coverage %
A+C	1979	3m - 19y	4 149801	3 245046	78,2
VA-MENGOC-BC®	1988-1990	3m - 5y	1 030000	887948	86,2
		6 - 14y	1 393900	1 050483	75,3
		15 - 19y	1 149000	754015	65,6
		Total	3 572900	2 692446	75,38

it work at least in mice. For more results see González E et al. in this edition (13).

Fig. 1. Morbidity of *Neisseria* pathogenic species since 1970 in Cuba



In conclusion, epidemiological data and experimental results suggest the presence of mucosal cross recognition between *N. meningitidis* serogroups and *Neisseria* species which could be involved in Gonorrhea protection. The use of nasal AFCo1 to obtain immunity against both strains may be a useful strategy to combat both infections in humans. Single Time Vaccination Strategy could be important to increased human vaccination coverage and herd immunity protecting both systemic and mucosal environments.

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