# Nasal immunization with AFCo1 induces immune response to *N. gonorrhoea* in mice

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*Neisseria gonorrhoeae* infections are common sexually transmitted diseases. Increased antibiotic-resistant of *N. gonorrhoeae* strains were reported. *N. meningitidis* is another human restricted bacterium transmitted through mucosa. However, the induction of systemic specific IgG antibody against some proteins between the two species is known, but the mucosal immune response to these pathogens is not clear. We hypothesized that *N. meningitidis* could induce immune response against *N. gonorrhoeae*. Therefore, serogroup B Proteoliposome (PL) was transformed into AFCo1 (Adjuvant Finlay Cochleate 1) and used for nasal immunization of C57Bl/6 mice. The specific IgG and IgG subclasses against both antigens in sera and vaginal extraction were measured by ELISA. Specific proliferation ($^3$H incorporation) of spleen cells and lymph node recall *in vitro* with PL or *N. gonorrhoeae* total antigens was measured. Serum and vaginal extraction anti *N. meningitidis* and *N. gonorrhoeae* IgG as well as the induction of specific IgG subclasses were detected. *N. gonorrhoeae* induces specific proliferation of spleen, cervical lymph node (cLN), and mediestinal (meLN) cells from immunized mice. In conclusion, AFCo1 induce anti *N. meningitidis* immune responses that recognized *N. gonorrhoeae* antigens in mice.

## Introduction

The World Health Organization report estimated that there were 62.2 million cases of the sexually transmitted infection gonorrhoea worldwide (1). Antibiotics are the treatment of choice for Gonorrhea, but the increasing emergence of drug-resistant strains has made treatment more difficult and expensive (2). On the other hand, approximately 10% of the healthy population are colonized with *N. meningitidis* in the nasopharynx but only rarely develop disease (3), but *N. meningitidis* carriage leads to the development of protective immunity both at the mucosal surface and systemically (4,5).

Mucosal vaccine delivery is a promising strategy, particularly because mucosal vaccines administered in one part of the body can elicit an antibody response in mucosal tissues remote from the site of initial antigen exposure (6). The mucosal immune system is uniquely structured for the development of effective immune responses against pathogens that invade mucosal surfaces. The administration of immunogenic formulations through mucosal (intranasal, oral, intravaginal, or intrarectal), routes is likely the best approach of inducing immune responses in both systemic and mucosal immune compartments.

Meningococcal vaccine, VA-MENGOC-BC® an effective parenteral vaccine against *N. meningitides* serogroup B was developed in Cuba (7). This vaccine has in its composition many Outer Membrane Proteins, in form of Proteoliposome (PL), as principal antigenic components for protection against serogroup B. In addition, some structural similarities between these proteins and *N. gonorrhoeae* proteins have been reported (8).

Previous studies have demonstrated that i.n. immunization of mice with AFCo1 and PL induced a strong IgG response in sera against PL antigens, significantly higher in AFCo1 immunized groups. Therefore, we though AFCo1 applied by IN route will be able to induce cross immune responses against *N. gonorrhoeae* in mice.

## Materials and Methods

PL was produce by Finlay Institute from serogroup B *N. meningitidis* strain cu385-83 AFCo1 was obtained from PL as previously described (9). Female C57Bl/6 mice (Taconic M&B, Denmark), were inoculated three times intranasal (IN) route with AFCo1. Then, sera and vaginal extraction were collected. Antibodies specific IgG response in sera and vaginal extract of immunized mice were determined by ELISA (10). The supernatants of unpurified spleen cells or lymph node culture recall *in vitro* with PL or total *N. gonorrhoeae* antigens. The cells were pulsed with 1mCi of thymidine [$^3$H] (Amersham, Pharmacia) and then assayed by liquid scintillation counting. Statistical analyses were done by Student's *t*-test using Graph Pad Prism 4 software (CA, USA).

## Results

In this study, we investigated if the AFCo1 is able to induce antigen specific systemic immune responses. Mice immunized by IN route showed higher titter of specific anti *N. meningitidis*

and *N. gonorrhoeae* IgG in sera and in the vaginal extracts, compared with the mice that not received the AFCo1 (Figure 1 and 2). Total spleen cells and cells of (cLN) and (meLN), but not the cells from genital (g) LN, from immunized mice, showed significantly higher tittres of proliferative responses against *N. meningitidis* and *N. gonorrhoeae* compared with cells isolated from not immunized mice ($p < 0.01$) (Figure 3 A and B). The AFCo1 induced a potent cell proliferation against *N. meningitidis* and *N. gonorrhoeae* in samples evaluated using IN immunization.

## Discussion

The administration of vaccines to mucosal surfaces would confer considerable advantages since mucosal surfaces are the sites through which most antigens are encountered. Previous studies have shown that IN immunization is an effective means for the induction of serum and mucosal antigens specific antibodies. The prolonged induction of genital tract antigen-specific antibodies following IN vaccination has highlighted this route of immunization as an attractive potential method for preventing sexually transmitted infections (11). We immunizing mice IN with AFCo1 and demonstrated that this immunization is an effective means of eliciting specific serum and vaginal anti *N. meningitidis* and *N. gonorrhoeae* IgG antibodies.

## Conclusion

In conclusion, AFCo1 induce anti *N. meningitidis* and anti *N. gonorrheae* immune responses in mice that could be exploited for a bivalente vaccine design.

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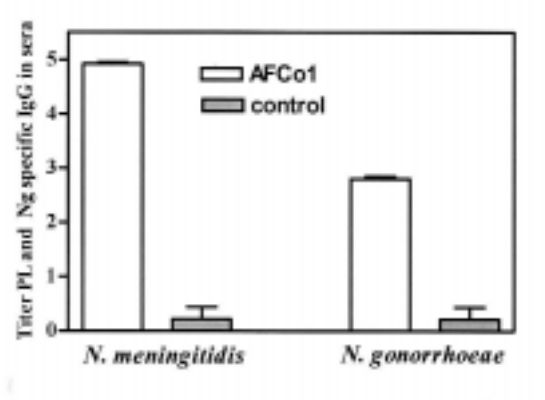


**Figure 1**. Serum cross recognition of *N gonorrhoeae* antigen by AFCo1 nasal immunized mice. Titter of specific IgG anti PL (*N. meningitidis* serogroup B) and anti *N. gonorrhoeae* (Ng) obtained in serum of animals immunized with nasally with AFCo1.

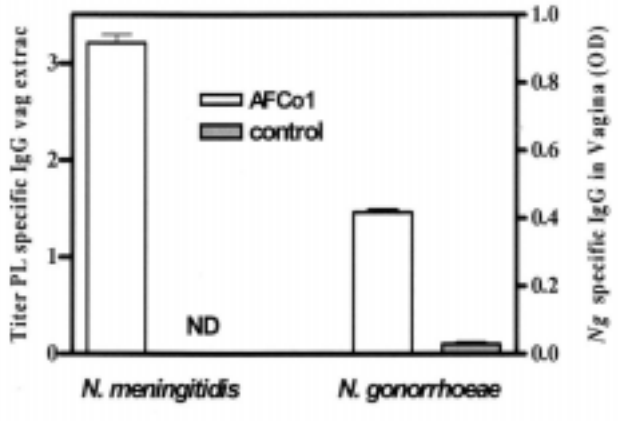


**Figure 2**. Vaginal cross recognition of *N gonorrhoeae* antigen by AFCo1 nasal immunized mice. Titter of specific IgG anti PL (*N. meningitidis* serogroup B) and anti *N. gonorrhoeae* (Ng) obtained in vaginal extracts of animals immunized nasally with AFCo1.

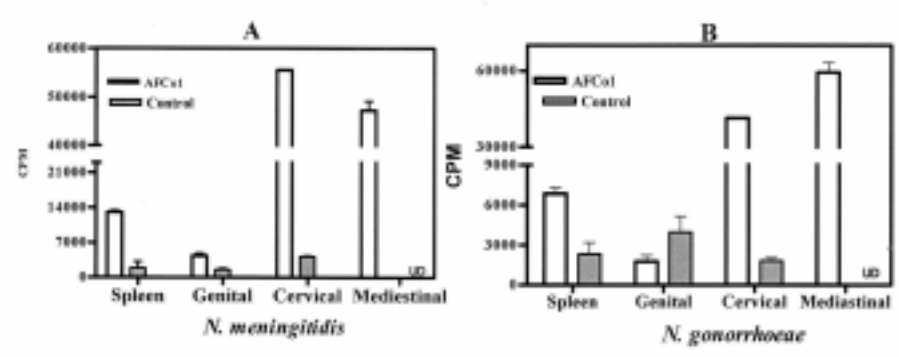


**Figure 3.** Specific proliferative in spleen, gLN, cLN and meLN cells after nasal immunization with AFCo1. Groups of female C57Bl/6 mice (n=7) were nasally vaccinated with AFCo1. Four weeks after immunization, genital, spleen, genital, cervical and mediestinal lymph node cells ($10^6$/ mL) were co-cultured with PL (A) and total N. gonorrhoeae antigens (B). The results are expressed as the mean + standard errors of counts per minute (cpm) for proliferation.

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