

## Proteoliposome and cochleate derived from serogroups B *N. meningitidis* for mucosal vaccines

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### Abstract

**Introduction.** The vast majority of pathogens enter or establish at mucosal surfaces but most vaccines for humans use are administered parenterally, for example, *Neisseria meningitidis* is a major causative agent of bacterial meningitis and fatal septicemia. Mucosal delivery also has the advantage of stimulating local, as well as systemic, immune responses, thus preventing bacterial colonization in naïve individuals and preventing dissemination of bacteria that breach the mucosal barrier. However, most antigens are poorly immunogenic when delivered at mucosal sites and typically require the use of mucosal adjuvants. Nevertheless, the availability of safe and effective adjuvants that function mucosally is the major limitation. Adjuvants have been extensively investigated for vaccine development, most of the traditional are of bacterial origin. **Objective.** Therefore, we were investigating the impact of mucosal immunization with the *Neisseria meningitidis* B proteoliposome (AFPL1, Adjuvant Finlay Proteoliposome 1) and its-derived cochleate (Co, AFCo1). They contain multiple PAMPs as immunopotentiators, have delivery system capacities, and Th1 polarization activity. **Method.** Groups of female Balb/c mice were immunized by nasal, oral, intravaginal, or intramuscular routes with three doses with AFPL1 or AFCo1 alone and containing glycoprotein (g) D2 from Herpes Simplex Virus type 2 (HSV-2). **Results.** High levels of specific IgG antibodies were detected in sera in all evaluated routes and specific IgA antibodies were produced in saliva and vaginal wash only following mucosal delivering. The polarization to a Th1 pattern was confirmed by the induction of IgG2a/IgG2c antibody, positive delayed-type hypersensitivity reactions, and  $\gamma$ IFN production. Additionally, AFCo1gD2 showed practically no vaginal HSV-2 replication and 100% protection against lethal vaginal HSV-2 challenge. **Conclusion.** The results support the use of AFCo1 as potent Th1 adjuvant for mucosal vaccines, particularly for nasal route.

### RESULTS

#### AFCo1 or AFPL1 works as an adjuvant with a relevant antigen (gD2 of HSV-2)

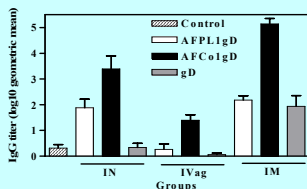


Figure 1. Mice immunized with AFCo1 or AFPL1 containing recombinant gD2 Shows high levels of anti-gD IgG in sera. The data are expressed as the geometric mean titer + standard errors (SE) of the means. Significant differences between the means of different groups were determined by a One-way ANOVA followed by Tukey's multiple comparison test.

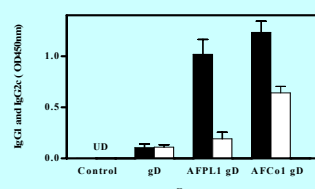


Figure 2. Shows the different IgG subclasses determined in sera. The data are expressed as the OD of samples measured at 450 nm + standard errors of the means. UD, undetectable values.

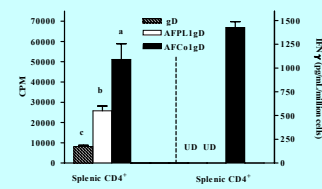


Figure 3. Shows gD-specific proliferation expressed as the mean of counts per minute (cpm) + standard deviations and the IFN- $\gamma$  concentration in co-cultures' supernatants. Significant differences between the means of different groups were

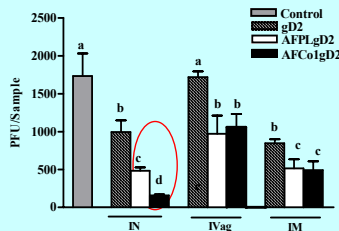


Figure 4. Mice immunized with AFCo1 or AFPL1 containing recombinant gD2 show low virus replication after HSV-2 challenge. Significant differences between the means of different groups were determined by a Tukey multiple comparison test using the Graph Pad Prism 4 software (Calif.).

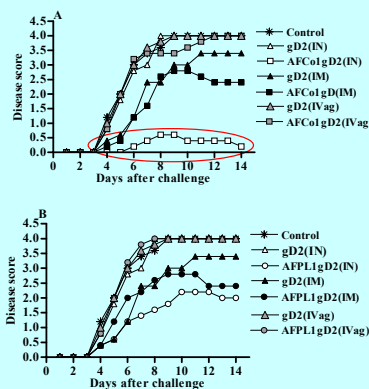


Figure 5. Developed macroscopic signs of disease was less in immunized groups for different routes with AFCo1(A) or AFPL1(B) containing recombinant gD2

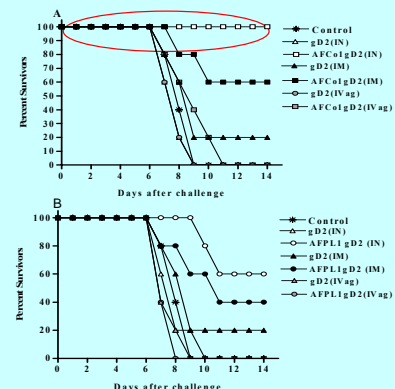


Figure 6. Immunization with AFCo1(A) or AFPL1(B) containing recombinant gD2 confers protective immunity against genital HSV-2 infection in mice

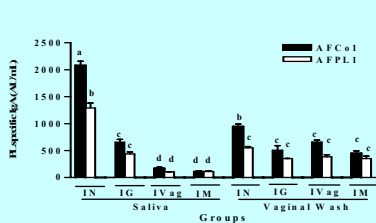


Figure 7. PL specific IgA in saliva and in vaginal wash responses induced by AFCo1 or AFPL1 administered by different routes. Significant differences between the means of different groups were determined by a Tukey multiple comparison test using the Graph Pad Prism 4 software (Calif.).

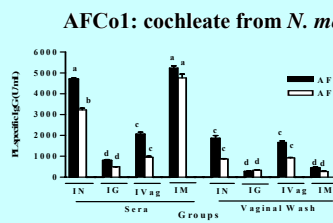


Figure 8. PL specific IgG in sera and in vaginal wash induced by AFCo1 administered by different routes

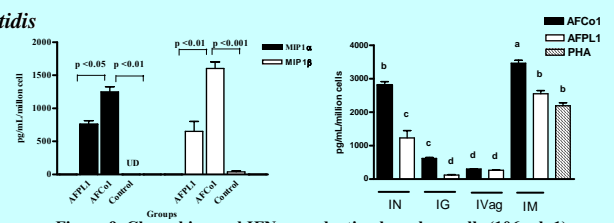


Figure 9. Chemokine and IFN- $\gamma$  production by spleen cells (106 ml<sup>-1</sup>) re-stimulated in vitro. Significant differences between the means of different groups were determined by a Tukey multiple comparison test using the Graph Pad Prism 4 software (Calif.).

### CONCLUSIONS

- Structural transformation of AFPL1 to AFCo1 increase the immune response
- AFCo1 containing OVA immunized group displayed considerable amounts of IgA, IgG and IgG subclass
- Nasal immunization with AFCo1 containing gD2 has been shown to induce better mucosal and systemic immunity
- Intranasal immunization with AFCo1 containing recombinant gD2 protein confers total protective immunity against genital herpes infection in mice
- AFCo1 have the capacity to enhance the immune response against the proteoliposome antigens
- AFCo1 has proved to function very well both as nasal vaccine *per se* and as a mucosal adjuvant for nasal vaccine such as HSV-2, human immunodeficiency virus, and human papilloma virus.