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Mucosal and systemic response induced by intranasal immunisation of C polysaccharide of *Neisseria meningitis* C and AFCo1

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ABSTRACT

Simple administration of antigens at mucosal sites have demonstrated be inefficient to stimulate the immune system by the weak immunogenicity inherent of these antigens. The use of mucosal adjuvants is capable to change this problem, increasing the mucosal and systemic immune responses. AFCo1 had demonstrated be a potent mucosal adjuvant with thyme-dependent antigens. This work demonstrated that intranasal immunisation (i.n) of polysaccharide C (poli C) of *Neisseria meningitidis* and AFCo1, as mucosal adjuvant, with 3 doses in Balb/c mice promote higher statistics specific IgA anti-poli C responses (p<0.001) in saliva and vaginal wash than the i.n immunization of poli-C and VAMENGOC-BC by the intramuscular route (i.m). Also, were induced a statistics significant specific IgG response anti-poli C (p<0.05) superior to i.n poli-C, but not different to i.m VAMENGOC-BC. The subclasses pattern and the IgM response were analyzed. The long-time mucosal and systemic memory responses were evaluated, after the booster immunization with poli-C 70 days later of the last immunisation. These results suggest that AFCo1 is a potent mucosal adjuvant not only for thyme-dependent antigens, also to thyme-independent antigens type 2 and is capable to induce memory responses.

INTRODUCTION

Vaccines made from protein antigens have been very successful in the induction of protective immune responses. However, the development of protective vaccines against capsular polysaccharides from pathogens like: *Neisseria meningitidis, Haemophilus influenza o Streptococcus pneumoniae,* has encountered many difficulties. Polysaccharides (PS) molecules carry repeated carbohydrate residues and they are representative of the class of antigens referred as T-cell independent type-2 (TI-2). In general they are poor inducers of immunological memory, and are able to induce rapid responses characterized by the production of IgM (1).

Oral and nasal mucosal immunizations have received a great deal of attention in recent years. Mucosal immunizations stimulate both mucosal and systemic immunities, and antigen-specific mucosal immune responses can be expressed at all mucosal sites. Subunit vaccines require adjuvants in order to promote strong and prolonged mucosal, local and systemic immune responses, but the only adjuvants widely approved for human use, aluminium salts, are not very effective (2).

Neisseria meningitidis outer membrane vesicles (OMV, Proteoliposome, PL) has been widely used as a core antigen for meningococcal vaccination in the Cuban using Meningococcal BC vaccine VA-MENGOC-BC™ (2). The structural transformation of the PL into cochleate structures (AFCo1) provides additional properties to PL. AFCo1 had demonstrated be a potent mucosal adjuvant (3,4), a microparticle that showed high stability, antigen delivery capacity and contain multiple PAMPs as immunopotentiators (5).

The aim of this work was to demonstrate the capacity of AFCo1 to induce systemic and mucosal response using a TI-2 antigen as poli C. In parallel, we evaluated the capacity to induce a long time mucosal and systemic memory response against poli C.

MATERIALS AND METHODS

Antigens: Poli C and PL of the epidemic meningococcal strain Cu385 (B:4:P1.19,15) were supplied by the manufacturing plant and the development plant of Finlay Institute, Cuba. AFCo1 was prepared from this PL by rotational dialysis (4).

Mice: Female BALB/c mice (6-8 weeks old and weighing 18-20 g) were obtained from The National Center for the Production of Laboratory Animals, Havana, Cuba and housed in the Animal Care Unit during the experiments.

Vaccination study: Female mice were immunized with AFCo1 coadministrated with poli C (10 µg) by i.n route in 3 doses, seven days apart. Long time mucosal and systemic memory responses, using the classic schedule of 3 doses and 70 days later a boost immunisation with poli C alone were evaluated.

Collection of samples. Saliva samples were collected 7days after the last dose meanwhile serum samples and vaginal secretion were collected 21 days after the last dose.

Analysis antibodies responses: Antibodies specific titers (IgG, IgA, IgM and IgG subclasses) in sera, saliva, and vaginal wash of immunized mice were determined by capture enzyme-linked immunosorbent assay (ELISA) (5).

Statistical analysis. Significant differences between the means of different groups were determined by a Tukey multiple comparison test using the Graph Pad Prism 4 software (CA, USA).

RESULTS

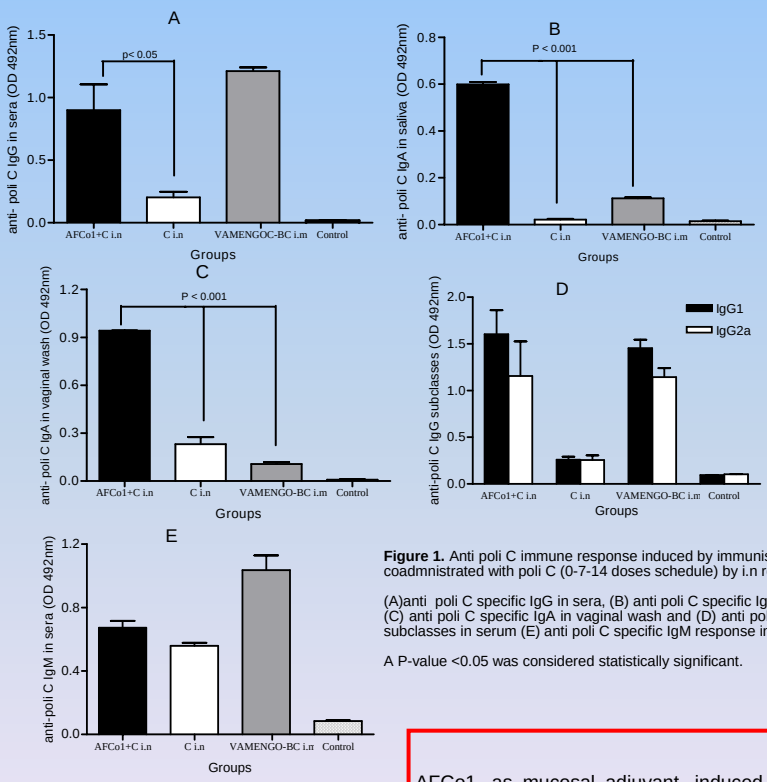

Figure 1. Anti poli C immune response induced by immunisation of AFCo1 coadmnistrated with poli C (0-7-14 doses schedule) by i.n route.

(A)anti poli C specific IgG in sera, (B) anti poli C specific IgA in saliva
(C) anti poli C specific IgA in vaginal wash and (D) anti poli C specific IgG subclasses in serum (E) anti poli C specific IgM response in sera.

A P-value <0.05 was considered statistically significant.

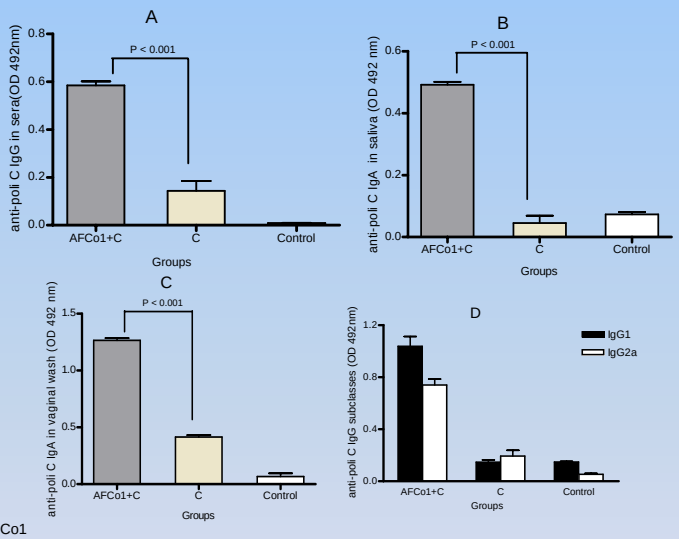

Figure 2; Anti –poli C primary response and long time memory response induced by a booster dose of poli C (10 µg) in saline by nasal route.

(A)anti poli C specific IgG in sera, (B) anti poli C specific IgA in saliva
(C) anti poli C specific IgA in vaginal wash (D) anti poli C specific IgG subclasses in serum
A P-value <0.05 was considered statistically significant.

CONCLUSIONS

AFCo1, as mucosal adjuvant, induced significantly (p<0.001) higher anti-poli C specific mucosal responses than those observed in the group received only poli C i.n and VAMENGOC-BC, the cuban vaccine, by the intramuscular route (i.m). AFCo1, coadministrated to poli C, induced higher anti-poli C IgG response (p<0.05) in serum, superior to i.n poli-C, but not different to i.m VAMENGOC-BC. AFCo1 induced higher long time mucosal and systemic memory responses (p<0.001) after a boost immunisation with poli C alone 70 days later. These results suggest that AFCo1 is a potent mucosal adjuvant not only for thyme-dependent antigens, also to thyme-independent antigens type 2 and is capable to induce memory responses

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