NATURAL *Neissera* DERIVE PROTEOLIPOSOME AND COCHLEATE AS POTENT VACCINE ADJUVANTS

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Summary

Proteoliposome (AFPL1) and its derivated - Cochleate (AFCo1) contain immunopotentiating and immunomodulatory properties and delivery system capacities required for a good adjuvant. In addition, they permit package of multimeric antigens and synergistic pathogen-associate molecular pattern essential to stimulate innate immunity. AFPL1 and AFCo1 is produce in GMP condition at industrial scale at Finlay Institute. Both, AFPL1 and AFCo1 contain protective anti *Neisseria* components. AFPL1 was used for several years as main adjuvant and antigen of Men BC combine vaccine where induces a preferential Th1 pattern of immune response. That is why, it is using in formulation against allergen to overcome the Th2 response induced by them. AFCo1 is more efficient that AFPL1 mainly for mucosal routes and a nasal vaccine is in preclinical stage of development. Preliminary results against Malaria and sexual transmitted disease are very promising. AFPL1 and AFCo1 acting directly as vaccines or used as adjuvants are very promising**.**

Key words: Adjuvants, Vaccines, Proteoliposome, Cochleate, *Neisseria*.

Proteoliposome (AFPL1) and its derivated - Cochleate (AFCo1) contain immunopotentiating and immunomodulatory properties and delivery system capacities required for a good adjuvant permitting the package of multimeric antigen and synergistic pathogen-associated molecular patterns (PAMP). Consequently, we hypothesized that AFPL1 and AFCo1 will function as good vaccine adjuvants.

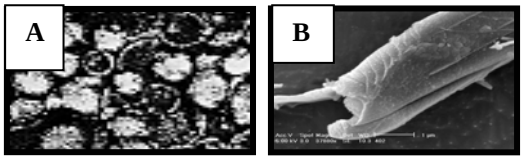
Methods

AFPL1 is a detergent-extracted outer membrane vesicle from live *Neisseria meningitidis* B and can be transforming into AFCo1 in calcium environment. Both are produced at industrial scale and control at Finlay Institute under GMP conditions[1,2,3,4].

Results

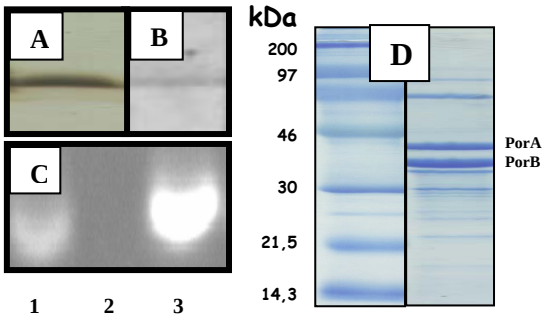
The AFPL1 is a self-assembling and non-replicating vesicle of around 70 nm (Fig 1A) and the AFCo1 is a macro-compact multilaminar tubular structure (Fig, 1B).

Fig. 1. Electronic micrograph of AFPL1 (A) and AFCo1 (B).



The immunopotentiator identified in the AFPL1 and AFCo1 in relationship to protein contain are: native LPS at 4±2% (Fig. 2A and B); PorA and PorB which represent the 65% (Fig. 2D); and trace of bacterial DNA at 0.016% (Fig. 2C).

Fig. 2. A, control LPS and B, AFPL1 LPS detected by monoclonal antibodies against L3,7,9. C, DNA (1, AFPL1 pelleted, 2 AFPL1 supernatant, and 3, control DNA), and D, AFPL1 SDS-PAGE right and MW left.



The Immunomodulator activity of AFPL1 and AFCo1 are mainly due to their main LPS PAMP. In mice without LPS signaling route (C3H/HeJ) the co-stimulation signaling (CD80, CD86, and CD40) and the Th1 response determine by IFNγ and serum IgG2a subclass production is decreased but not absent[5].

The delivery system is constituted by the capacity of lipid bilayer of AFPL1 and AFCo1 to interact with cell membrane. In addition, the calcium contain of AFCo1 add additional destabilizing properties to this microparticle having fusogenic properties and function without phagocytosis needed.

Discussion

AFPL1 and its AFCo1 have exceptional characteristics because they: (a) combine in the same structure the potentiator activity, the polarizing agents, and the delivery system capacities; (b) exhibit multimeric copies (repetition of porins and other proteins); (c) contain multiprotein composition (Hmbr, FrpB, PorA, PorB, rmpM, Opc, Opa, SpA, etc.); (d) contain multi PAMP components (LPS, porins, phospholipids, DNA, etc.); (e) contain synergistic PAMPs-TLRs interactions (TLR4 and TLR9); (f) act co-administrated but also allow the inclusion of other PAMPs, proteins, peptides, and plasmids; (g) induce both type I IFN and IL-12 cytokines which suggest the stimulation in human of plasmocytoid precursor and conventional dendritic cells, respectively; (h) induce a preferential Th1 pattern of response[(6)]; (i) induce T CD4+, T CD8+, and cross presentation[(5)]; (j) has polyclonal B cell activity, and (k) function by parenteral and mucosal routes[(7)].

The constitution of Proteo-Cochleate that contains protectogenic antigens permits *per se* their function as vaccines. The main example is the Cuban anti meningococcal vaccine[(1)] where the AFPL1 constitute it main immunopotentiator and antigens. The AFCo1 is concluding it preclinical stage to be use as a Nasal vaccine[(7)]. The inclusion of heterologous PAMPs and the formation of Cochleate using Proteoliposome from other microorganisms is a real fact. AFPL1, in addition to Men BC vaccine that is in Phase IV, has concluded the preclinical stage in an allergic vaccine[(8)].

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