CENTER FOR VACCINES RESEARCH & PRODUCTION
Ave 27, No. 19805, La Lisa, Havana City, Cuba. P.O. Box: 16017, Cod. 11600

Production of large scale of AFCo1 as Vaccine Adjuvant

Caridad Zayas, Domingo González, Reinaldo Acevedo, Judith del Campo, Miriam Lastre, Elizabeth González, Belkis Romeu, Maribel Cuello, Julio Balboa, Osmir Cabrera and Oliver Pérez.
Immunology Department, Vice presidency of Research, Finlay Institute.
Corresponding mail author: czayas@finlay.edu.cu

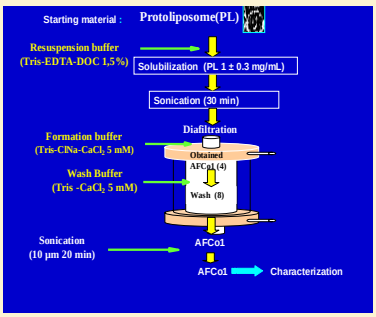
Abstract

The use of new adjuvants in vaccine formulations is a subject of current research. Only few parenteral adjuvants have been licensed. We develop a mucosal and parenteral adjuvant entitled AFCo1 (Adjuvant Finlay Cochleate 1) using dialysis procedures at lab scale. In order to increase the production process, we used a crossflow system (CFS) that allows easy scale up to obtain large batches in an aseptic environment. The aim of this work is to produce AFCo1 at pilot scale conserving adjuvanticity. Results. AFCo1 was successful produced by tangential filtration to scale pilot. It passes preclinical toxicity studies and preliminary stability. The nasal immunogenicity evaluated in Balb/c mice induced specific saliva IgA and serum IgG. The induction of Th-1 response was demonstrated by the induction of IgG2a, IFNγ and not IL-5. The self adjuvant action as well as against co-administered antigens were similar to Lab scale lots. The AFCo1 obtained at pilot scale conserve the mean physic-chemical and immunological characteristics for 2 years with no preclinical toxicity.

Introduction

Over the last decade, there has been a flurry of research on adjuvants for vaccines, and several novel adjuvants are now in licensed products or in late clinical stage development. The success of adjuvants in enhancing the immune response to recombinant antigens has led many researchers to re-focus their vaccine development programs. Successful vaccine development requires knowing which adjuvants to use and knowing how to formulate adjuvants and antigens to achieve stable, safe and immunogenic vaccines. In addition to the demonstration of safety, immunogenicity and protection in preclinical (where a model exits) and clinical studies, any Adjuvant System will need to demonstrate feasibility of large-scale manufacturing. This includes definition, selection, supply and characterization of the raw materials, as well as the development of production processes that will deliver a stable and consistent product. In order to reach the final product, manufacturing processes, analytical and immunological tools, and quality control testing need to be conducted successfully. Toxicology studies need to be designed on a case-by-case basis and performed with the adjuvant alone and in combination with the antigen of interest, according to the existing guidelines. All steps are very important for the delivery of reliable Adjuvant System to be produced at large scale. Only through this strict and controlled process will the quality of future adjuvant vaccines be guaranteed. Obtaining AFCo1 using tangential filtration process would ensure the production of a large scale adjuvant safe, immunogenic and non toxic.

Materials and Methods



Results

AFCo1 (Coclheate) obtained by Cross Flow System

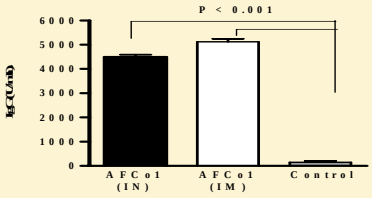


Fig. 1. PL specific IgG antibody response in sera. Balb/c mice were immunized with AFCo1 for intranasal (IN) and intramuscular (IM) routs. The sera were evaluated by ELISA

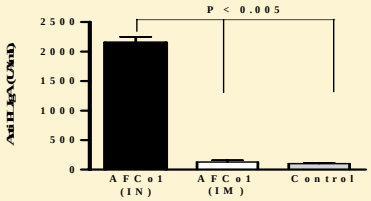


Fig. 2. PL specific IgA in saliva responses induced by AFCo1 administrated by IN and IM routes. Significant differences between the means of different groups were determined by a Tukey multiple comparison test using the Graph Pad Prism 4 software (Calif.)

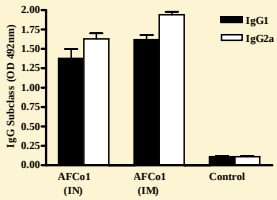


Fig. 3. PL specific IgG1 and IgG2a antibody response in sera

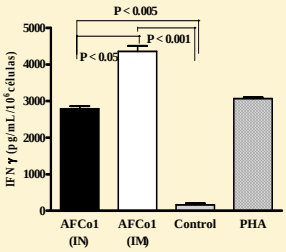


Fig.4. Cytokines secreted in cell re-stimulated in vitro with Proteoliposome (PL). Cells were either with PL (5 µg/mL) or PHA (10 µg/mL) as positive control. IFNγ levels (pg/mL) in the supernatants were determinate by ELISA for stimulation times and expressed as mean ± Standard deviation (SD) of triplicate cultures. One represent experiment of three is show (p< 0,005) by Turkey Test

AFCo1 obtained by Cross Flow System works as an adjuvant with using OVA as model antigen

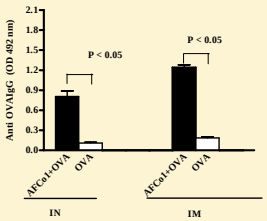


Fig.5. OVA specific IgG in sera responses induced by AFCo1 administrated by IN and IM rout

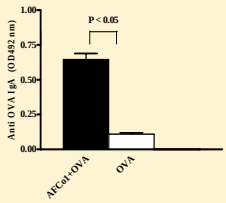


Fig. 6. OVA specific IgA in saliva responses induced by AFCo1 administrated by IN rout

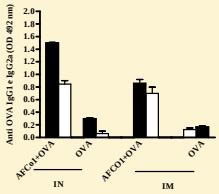


Fig.7. OVA specific IgG1 and IgG2a antibody responses induced by AFCo1 in sera

Results of stability and toxicity preliminaries

➢**The pre-stability study was realized for 48 months at 5 ± 30°C, which culminated in a satisfactory manner in compliance with the parameters measured. This process included particle size, shape, appearance, protein concentration, identity, microbial limits and preservative effectiveness of anti-PL IgG response in serum and IgA in saliva.**

➢**For toxicity testing, there was no evidence of systemic toxicity, regarded as non-toxic for human use.**

Conclusions

➢AFCo1 obtained by the methodology of cross flow ultrafiltration:
❖Produced highest levels of specific antibodies in serum and saliva by mucosal route
❖Have de capacity to enhance the Mucosal and Systemic response against the proteoliposome using OVA as model antigens
❖Tangential filtration process would ensure the production of a large scale adjuvant safe, immunogenic and non toxic