IMMUNOGENICITY POTENTIAL OF A NOVEL ANTIGEN-CARRIER SYSTEM

Silvia Moreno[1], Clara Espitia[2], Rogelio Hernández[3], Sergio Sánchez[1] and Romina Rodríguez[1]; Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, Departamentos 1. Biología Molecular y Biotecnología. 2. Inmunología. 3. INNCMSZ. Mexico city Z.C. 04510; moreno.sa@biomedicas.unam.mx

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Introduction. The association of proteins or peptides with polymeric microparticulated systems is a success strategy to carry and deliver antigens in mucosae. Not only to achieve properties such as retention of activity and prevention of enzymatic degradation (1), but also to induce strong immune responses (2).
In this work we use a Starch Binding Domain derived from *Lactobacillus amylovorus* α-amylase as tag for immobilization of fusion proteins on raw starch microparticles and their later mucosal administration.
To investigate the immunogenicity potential of this immobilization and carrier system, the protein alpha crystallin (Acr) from *Mycobacterium tuberculosis* was fused to the SBD, purified and immobilized on starch granules and the immune response characterized in BALB/c mice after oral and intranasal immunization.

Methods. Fusion protein Acr-SBD was produced in *Escherichia coli* and purified by beta cyclodextrin affinity chromatography (3). 50 and 100µg of protein were immobilized on starch and administered orally to female mice BALB/c on 3 consecutive days, every 21 days. Furthermore, 25 and 50µg of immobilized protein were intranasal administered on 2 consecutive days each 21 days. Immunization schedules are shown in following table.The immune response was characterized and compared to control group immunized with free Acr-SBD.

Oral		Intranasal	
Immunization days	Sampling days	Immunization days	Sampling days
1-2-3	4, 14	1-2	3, 14
21-22-23	24, 34	21-22	23, 34
41-42-43	44, 54	41-42	43, 54

Results. Oral and intranasal administration of Acr-SBD immobilized on starch microparticles induced stronger specific humoral responses in mice in comparison to animals immunized with free protein (Fig 1, 2). Immobilization also favored a Th1 response indicated by the IgG2a anti-Acr levels and corroborated by the antigen specific INF-γ detected in supernatants from splenocytes cultured and stimulated *in vitro* with 20µg of Acr and AcrDFA (Fig 3).

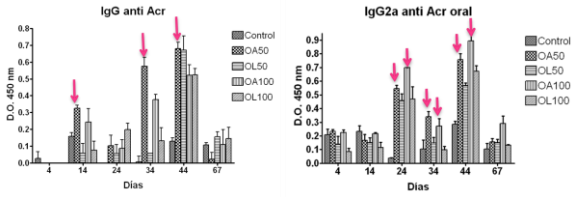

Fig.1 Anti-Acr specific IgG and IgG2a in sera of orally immunized mice determined by ELISA.

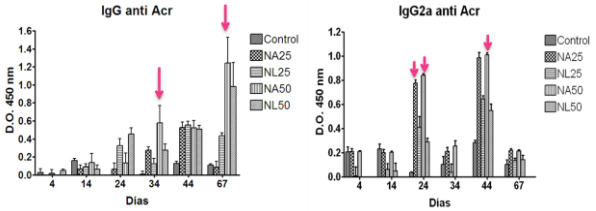

Fig.2 Anti-Acr specific IgG and IgG2a in sera of intranasally immunized mice determined by ELISA.

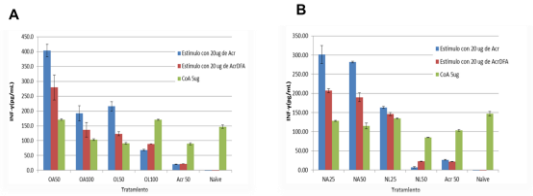

Fig.3 Antigen specific INF-γ released from splenocytes purified from **A.** orally and **B.** Intranasally immunized mice after *in vitro* stimulation.

Conclusions. Carrier and delivery system proposed has immunogenicity potential as determined by the serum levels of IgG anti Acr detected in mice immunized by both routes with the immobilized protein. It can even be considered a possible adjuvant effect as indicated by the subclass distribution of the systemic, humoral response (specific IgG2a) with the consequent cellular response activation (specific INF-γ).

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