STARCH GRANULES AS VEHICLE FOR ORAL ADMINSITRATION OF ANTIGENS

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Introduction. New vehicles for oral drug delivery are currently under study due to the advantages that this route presents over parenteral or subcutaneal administration. Microparticles are among the most studied vehicles for oral administration since their size improves the capture and/or presentation of peptides or proteins in the intestinal environment [1].
Starch is an abundant substrate, biocompatible, inexpensive, with an authorized use in food and pharmaceutical industry, as well as being naturally particulate, characteristics that make it ideal for use as oral vehicle for therapeutic protein and antigens.
Using an amylase starch-binding domain (SBD) [2] fused to the fragment C of tetanus toxin (Tc) as model antigen, the fusion protein TcSBD was produced, purified and adsorbed on starch granules. These inmobilized proteins were orally administered with the purpose of analyze and evaluate the systemic response in mice.

Methods. The fusion protein TcSBD was produced intracellulalyr in *E. coli* BL21 and purified from bacterial lysate through beta- cyclodextrin affinity chromatography using the SBD as a purification tag [3]. The purified protein was adsorbed, through the SBD, on rice starch and orally administered to six to eight weeks female mice Balb/c. Mice received TcSBD free and adsorbed to starch in two doses (25-75μg) and immunization schemes (Fig1). Blood samples were analyzed for IgG on immunoblots and ELISA assays.

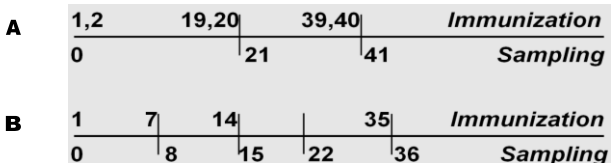

Fig.1 Immunization schemes used in this study.

Results. Mice who received orally TcSBD adsorbed to starch granule were capable to induce a specific humoral response stronger than mice who received TcSBD in free form.
In immunization scheme A, mice administered with 25μg of starch adsorbed TcSBD showed the greater IgG response in all the groups (Fig 2 and 3).

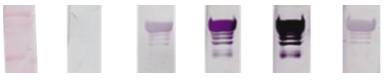

Fig. 2 Immunoblot with sera from groups immunized with TcSBD soluble (sol) and adsorbed (ads). 1)MM; 2)Control; 3)25 μg sol; 4)75 μg sol; 5)25 μg ads and 6)75 μg ads.

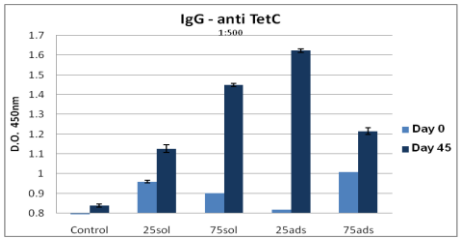

Fig. 3 IgG determination in sera of groups immunized with TcSBD soluble (sol) and adsorbed (ads) Scheme A.

Conversely, mice in immunization scheme B who received 75μg of TcSBD adsorbed to starch showed the highest IgG response, with virtually lesser response in mice that received the soluble antigen (Fig 4 and 5).

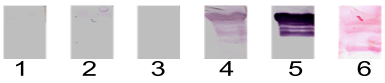

Fig. 4 Immunoblot with sera from groups immunized with TcSBD soluble and adsorbed. 1. Control; 2. 25 μg soluble; 3. 75 μg soluble; 4. 25 μg adsorbed; 5. 75 μg adsorbed and 6. MM

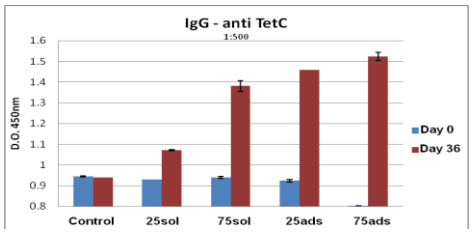

Fig. 5 IgG determination in sera of groups immunized with TcSBD soluble (sol) and adsorbed (ads) Scheme B..

Conclusions.

Starch immobilization through the SBD allows antigen delivery in mucosal associated lymphoid tissue
The antigen adsorption to starch through the SBD system enhances the IgG specific response in mice.
The immobilization allows the use of lower antigen doses for immunization.
The data shows that starch/SBD system can be used as vehicle for oral protein or antigen administration

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References.

1. O'Hagan DT.. (1998) Adv Drug Deliv Rev. 34:305-20
2. Rodríguez-Sanoja, R., Morlon-Guyot, J., Jore, J., Pintado, J., Juge, N. & Guyot, J.P (2000). Appl. Environ. Microbiol. 66: 3350-3356.
3. Guillén, D., Moreno, S., Aguilera, P., Sánchez, S., Farres, A. and Rodríguez-Sanoja, R. (2013) Appl. Microbiol. Biotechnol. In Press