Dendritic cell targeting in cultured fish in Argentina for vaccine development

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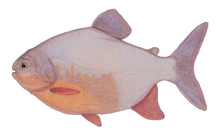
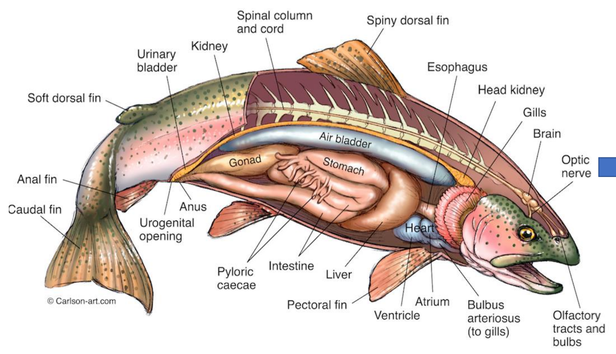
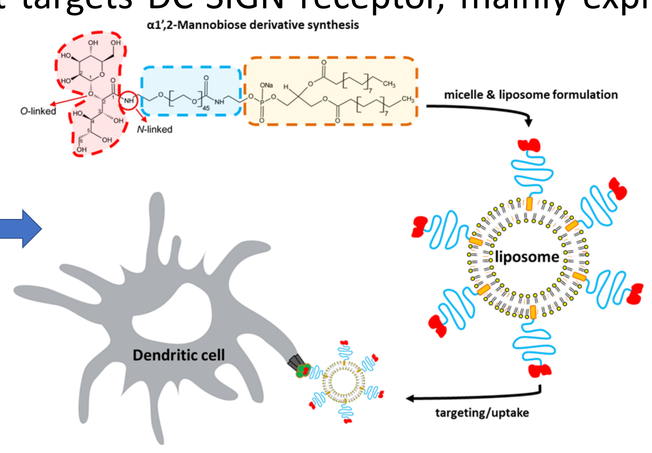


Introduction: In Argentina two main species present an important economic value, these are Pacú (*Piaractus mesopotamicus,* Pm) and Rainbow Trout (*Oncorhynchus mykiss,* Om). Due to stress and changing environmental conditions cultured fish are exposed to, the use of antibiotics has become a common solution for treatment and avoidance of disease. This practice presents several problems such as overdose, contamination and resistance generation. The development of effective and affordable vaccines is necessary for aquaculture in order to produce safe products for consumption and the environment.

Aim: The evaluation of a species unspecific nanovaccine platform in Pm and Om, composed of liposomes decorated with α1,2-mannobiose, a specific disaccharide that targets DC-SIGN receptor, mainly expressed on dendritic cells (DC).





Material and Methods: We cultured DC obtained from head kidney (HK), of Pm and Om in complete D-MEM (10% FBS) for 1, 7 and 14 days at room temperature (RT) in order to obtain non-adherent cells, enriched in DC. These cells where later incubated for 30m or 12h at RT in D-MEM without FBS with undecorated liposomes for unspecific cell targeting (plain-L), α1,2-mannobiose decorated (Manα-L) and DOTAP (DOTAP-L) liposomes as a positive control, all marked with rhodamine. Prior liposome formulation and characterization with ζ sizer was done. Incubation was stopped adding complete D-MEM. Cells were washed and fixed with PFA at 0.02% w/v final concentration and then analyzed by flow cytometry. Results were statistically analyzed with two-way ANOVA followed by Bonferroni's Test.

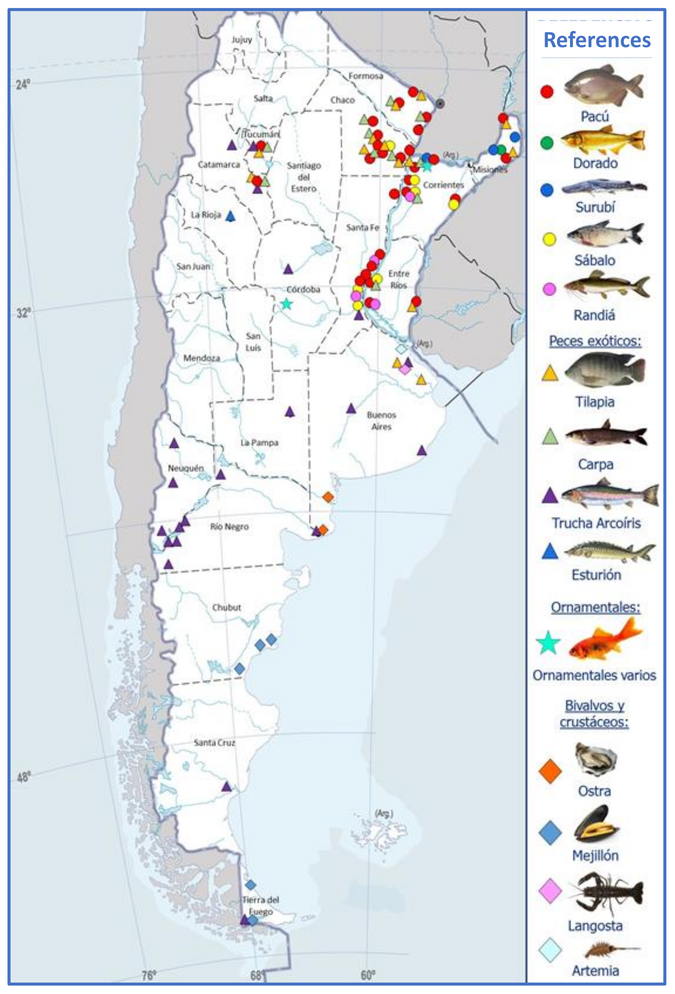


Figure 1. Map of cultured species in Argentinean aquaculture. Source: REFACUA.

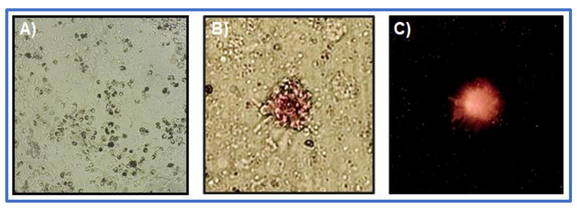


Figure 2. Dendritic cell cultures. A) representative photography of Om DC culture of day 2. B) Pm DC photography with light microscopy. C) Same picture frame as B) with flourescent microscopy.

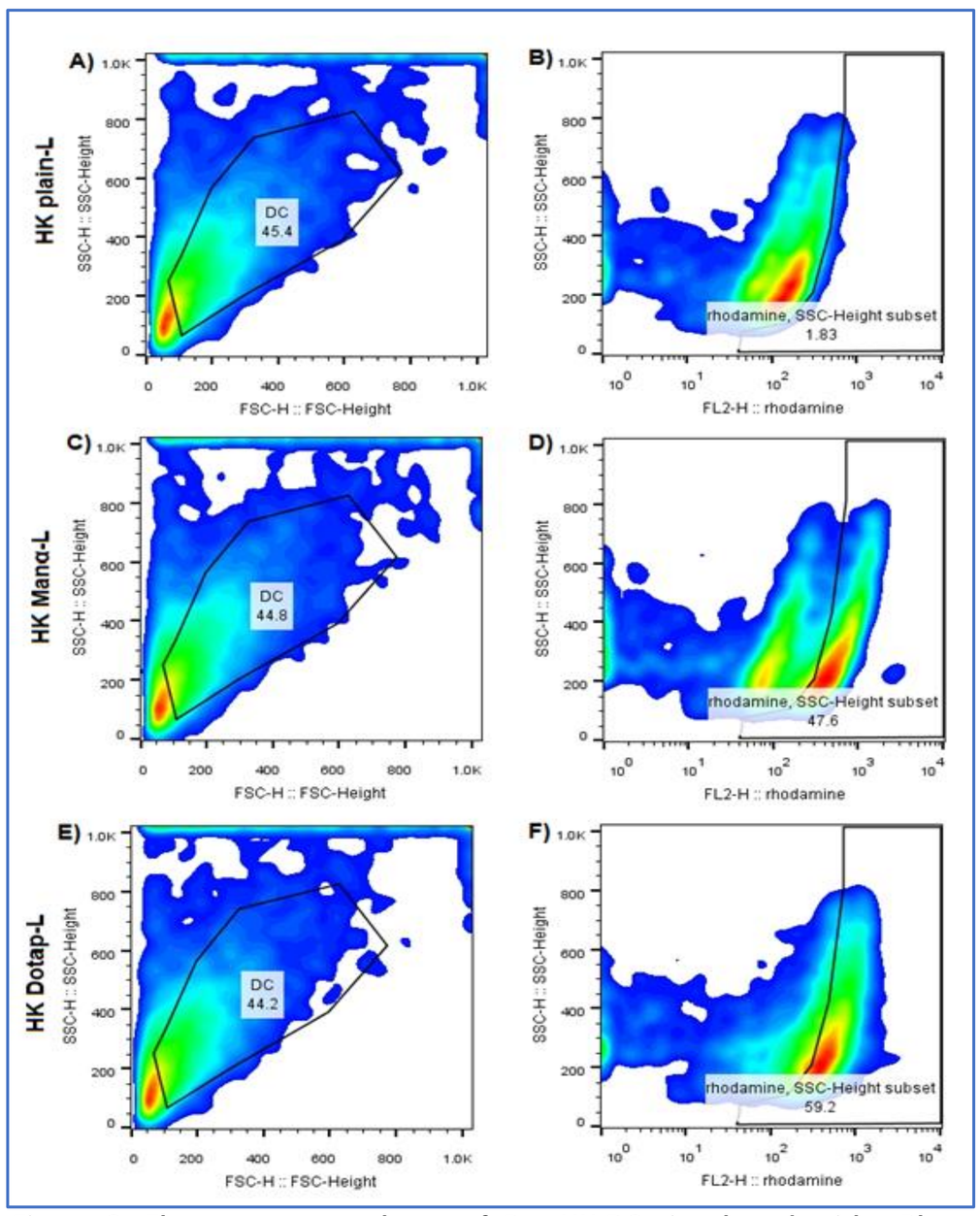
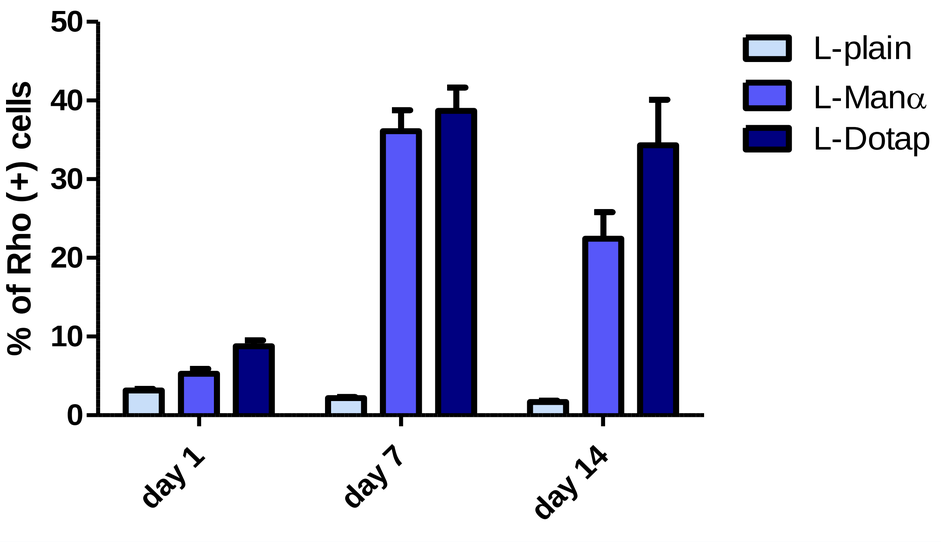


Figure 3. Flow cytometry charts of Pm HK-DCs incubated with Rho+ liposomes. A) & B) show cells incubated with plain-L. C) & D) show cells incubated with Manα-L, and E) & F) show cells incubated with Dotap-L, as a positive control. All liposome treatments were incubated for 30m.

Figure 4. Liposome HK cellular uptake quantification. Liposome treatments were incubated for 30m in cell cultures of 1, 7 and 14 days. Two-way Anova was performed indicating that differences between treatments and differences in culture age were extremely statistically significant, p<. 0.0001.



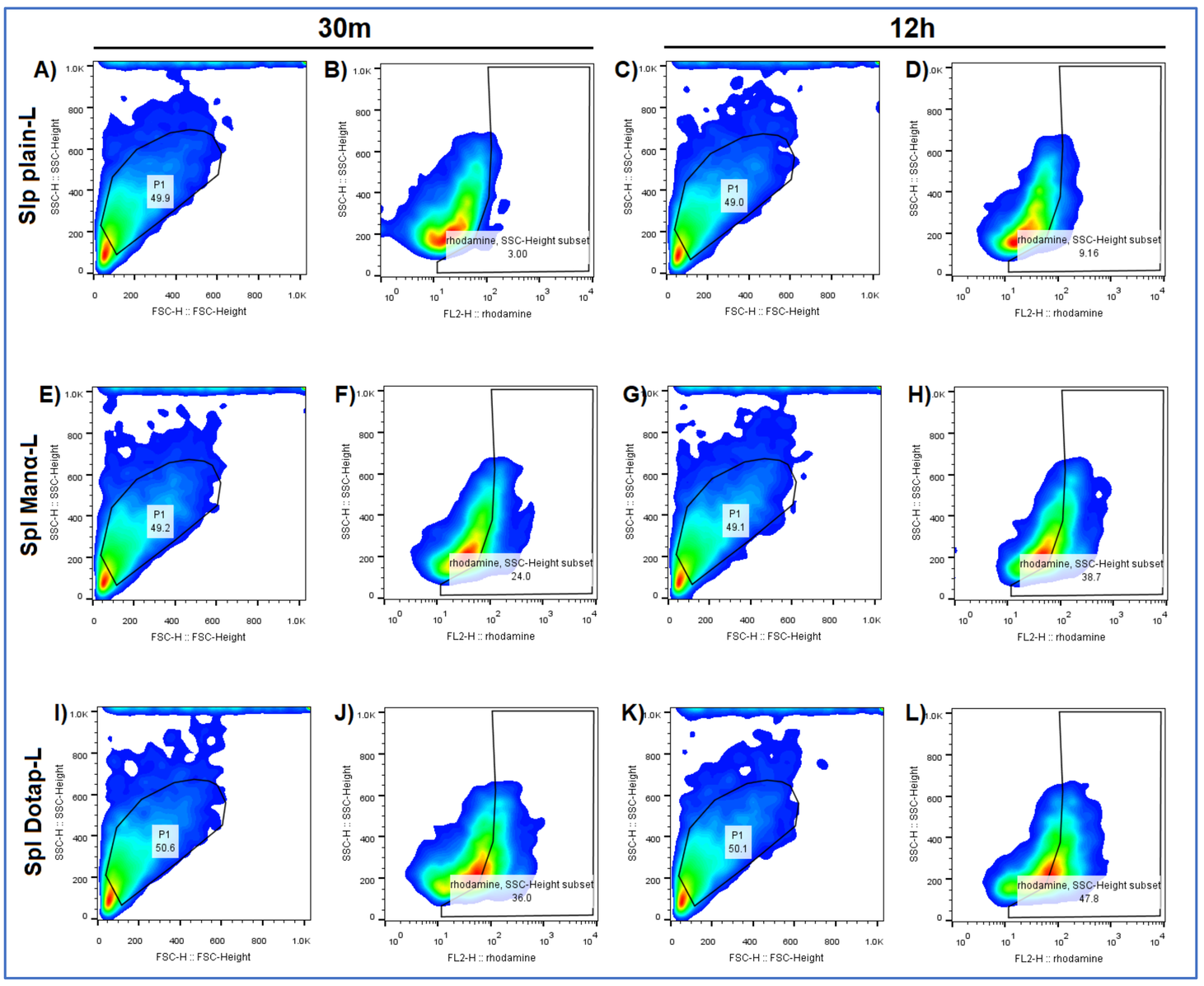
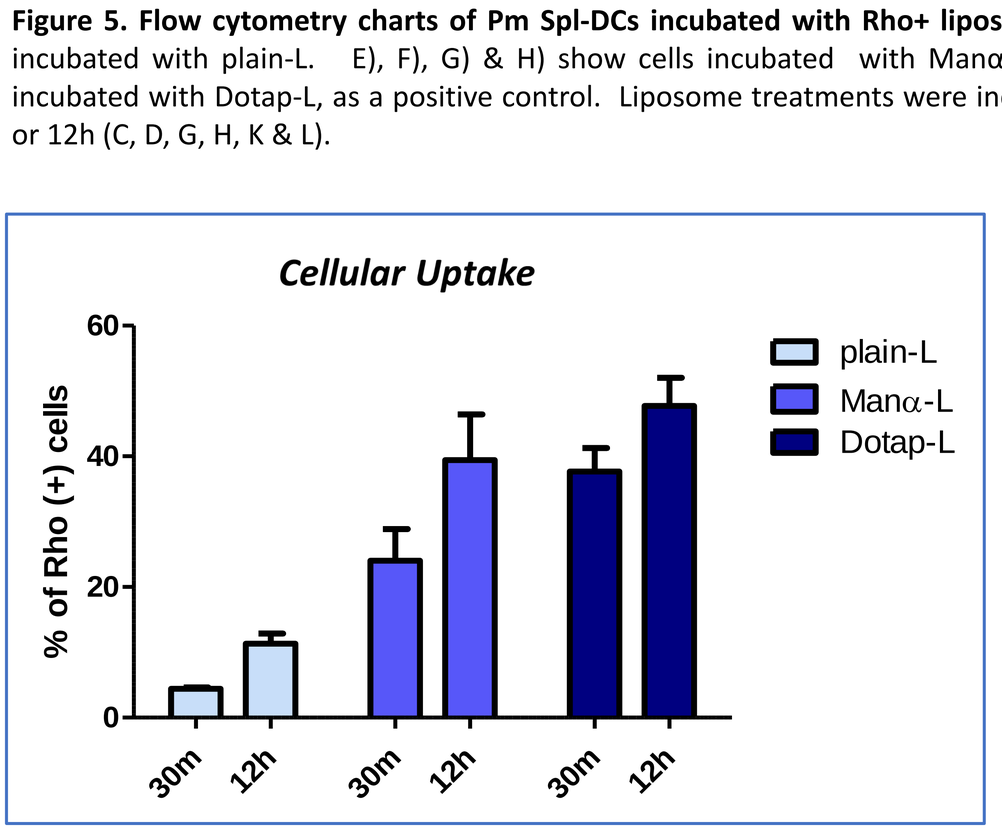


Figure 5. Flow cytometry charts of Pm Spl-DCs incubated with Rho+ liposomes. A), B), C) & D) show cells incubated with plain-L. E), F), G) & H) show cells incubated with Manα-L, and I), J), K) & L) show cells incubated with Dotap-L, as a positive control. Liposome treatments were incubated for 30m (A, B, E, F, I & J) or 12h (C, D, G, H, K & L).



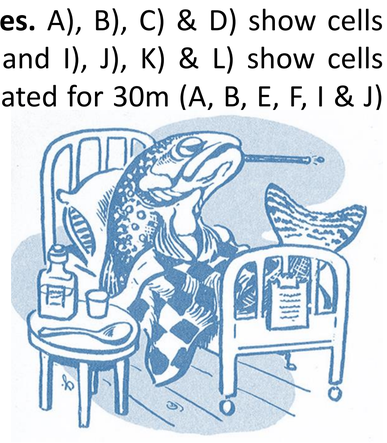


Figure 6. Liposome Spl cellular uptake quantification. Liposome treatments were incubated for 30m or 12h in cell cultures of 14 days. Two-way Anova was performed indicating that differences between treatments and differences in culture age were statistically significant, p<. 0.001.

Results: Here we demonstrate that HK cultures at day 7 and 14 are enriched in DC-SIGN expressing cells, and Manα-L targets specifically these cells (***p<0.0001).

Conclusion: These preliminary results indicate that the nanovaccine platform would be efficient in targeting DC, therefore could be an important tool in aquaculture vaccine development. Moreover, this platform can also be exploited as a specific DC dye in fish.