

VAC_17 - Intranasal/subcutaneous prime-booster immunization with Outer Membrane Vesicles of *Meningococci C* elicits high-avidity, persistent antibodies against *Meningococci B*

Amanda Izeli Portilho^{1*}; Gabriela Trzewikowski de Lima¹; Gabrielle Gimenes Lima¹; Elizabeth De Gaspari¹.

¹Instituto Adolfo Lutz.

Introduction: *Neisseria meningitidis* causes Invasive Meningococcal Disease (IMD). Immunization should achieve a persistent immune response against pathogens, at local and systemic levels, which can be modulated by administration site and appropriate adjuvants. Dimethyldioctadecylammonium bromide is a cationic lipid with adjuvant properties that can be used in as vesicles (DDA) or bilayer fragments (DDA-BF). Prime-booster scheme induces local and systemic antibodies.

Objective: Testing the persistence of antibodies triggered by immunization with outer membrane vesicles (OMV) of *N. meningitidis* C:4:P1.15, complexed with DDA or DDA-BF, against homologous and B:4:P1.15 strain, representative of the last epidemic period of IMD in Brazil.

Methodology: Antigenic preparations containing 0.25µg OMV+0.1mM DDA; 0.25µg OMV+0.01mM DDA-BF; 0.25µg OMV; 0.1mM DDA or 0.1mM DDA-BF were intranasally administered in adult Swiss mice on days 1, 2, 22 and 23. A subcutaneous booster containing 5µg OMV+0.1mM DDA; 5µg OMV+0.01mM DDA-BF, 5µg OMV; 0.1mM DDA or 0.1mM DDA-BF was administered on day 41. Blood was taken before immunization (pre-immune) and 180 days after the booster dose. ELISA plates (MaxSorp, Nunc) were coated with whole cells suspensions of strains C:4:P1.15 or B:4:P1.15 at an optical density (OD) 0.1 at 620nm. Serum samples at 1:50 were incubated for 2 hours at 37°C. Anti-IgG γ chain (Kirkegaard & Perry Laboratories) at 1:20,000 was incubated for 2 hours at 37°C. The reaction was revealed with the addition of tetramethylbenzidine for 20 minutes at 37°C, stopped with 1N sulfuric acid and read at 450nm (Molecular Devices). Results were analyzed using One-Way ANOVA method followed by Tukey's post-test (GraphPad Prism 8). The Avidity Index (AI) was performed by a modified ELISA using 1.5M potassium thiocyanate (KSCN) as a chaotropic agent and expressed as the ratio between OD with KSCN/without KSCN and considered high if AI>0.5, intermediate if . 0.5>AI>0.3 or low if AI<0.3.

Results: Against C:4:P1.15 strain, higher ODs were observed in immune samples when compared to the pre-immune and adjuvant controls, but there was no statistical difference between the groups. For B:4:P1.15 strain, when compared to the pre-immune control, the groups OMV (p=0.0342) and OMV+DDA-BF (p=0.0219) showed a statistical difference. However, all the individuals of OMV+DDA; OMV+DDA-BF and OMV groups showed high AI against C:4:P1.15, as well against B:4:P1.15, except for one individual from the OMV+DDA group who had intermediate avidity.

Conclusion: The immunization scheme was able to induce a persistent humoral response, with antibodies capable of binding to strains C:4:P1.15 and B:4:P1.15 with high AI. DDA-BF seems better than DDA as adjuvant. The group immunized with OMV showed a similar response to the groups with adjuvants in the preparation, suggesting that the strain itself is immunogenic. Functionality assays, as serum bactericidal activity should be enrolled to verify the protective potential.

Keywords: *Neisseria meningitidis*; Dimethyldioctadecylammonium bromide; Prime-booster immunization