Title: Development, Scale up and production of new and less reactogenic whole cell pertussis vaccine and an adjuvant in an integrated process: one approach to developing countries

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Abstract:

The association of adverse events in immunization with Whole cell Pertussis Vaccine (wP) has led to the development of Pertussis Acellular Vaccine (aP). However, it is known that immunization with aP does not have the same efficiency as compared to immunization with wP vaccine. Adverse reactions of wP vaccine are attributed to the presence of lipopolysaccharides (LPS) major constituents of the outer membrane of Gram-negative bacteria. Butantan Institute developed a vaccine, Pertussis low (Plow) with a reduced amount of LPS, which is less reactogenic than traditional cellular pertussis vaccine. Its production cost is lower than the aP vaccine and seems to have the same immunogenicity of traditional whole cell vaccine. In addition, a byproduct of this process is a solution that contain LPS, that can be transformed to become a potent adjuvant, the monophosphoryl lipid A (MPLA). To scale up the Plow production we evaluated three different process: tangential flow filtration (TFF), tangential filtration with organic solvent washing (TFFW), and Industrial centrifugation. These methodologies were compared with Plow produced at bench scale obtained by centrifugation and traditional wP. The LPS reduction was 25%, 50% and 80% in vaccines produced respectively with TFF, TFFW and centrifugation methods compared with wP. The centrifuged vaccine had the best endotoxin activity result (80% less). The major antigens were detected in all preparations and cell integrity was observed by electronic microscopy and flow cytometry. The extracted LPS from the pertussis cells were processed to generate MPLA. This adjuvant was emulsified at different concentrations (100 and 400 µg/ml) and analyzed for sterility, pH, toxicity, pyrogenicity, particle size, particle distribution and visual aspect. The MPLA-emulsions were sterile, atoxic, non-pyrogenic, 7.1-7.3 pH, monophasic aspect and the average particle size did not showed particle growth superior to 10% during 24 months, confirming its stability for at least 2 years. These results indicated the feasibility to produce Plow as a potential new whole cell pertussis vaccine for Industrial production as well as the MPLA as a byproduct. This adjuvant were tested with influenza vaccine allowing the reduction of antigen contain, consequently increasing the capacity of the influenza vaccine production. This approach that manufactures two product in an integrated process, lead to an affordable vaccine and adjuvant to the development countries.

Key-word: *Bordetella pertussis*, Scale up production, Vaccines production, Whole cell pertussis Vaccine, whooping cough.

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