

## Bacterial Vaccine Technology

### Outer membrane vesicles (OMVs)- Pertussis

- Surface components presented in natural conformation.
- Released spontaneously but can be induced by detergents, growth conditions, mutations.
- LPS as intrinsic adjuvant, but delicate balance immunogenicity vs. reactogenicity.

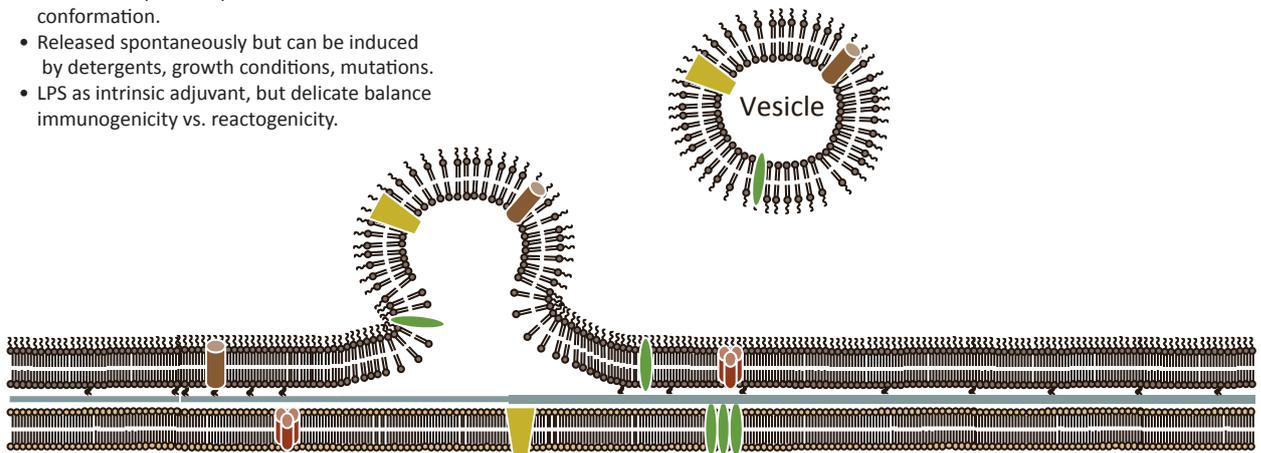


Figure 1: Release of OMV from the outer membrane of Gram negative bacteria. Gram-negative bacteria naturally release vesicles from the outer membrane. OMVs are spherical particles ( $\pm$  20-200 nm) that harbour many bacterial antigens, which play a role in establishing infection and survival of the bacterium within their host. Intravacc uses two approaches to develop OMV vaccines: homologous and heterologous. In this case, a homologous OMV vaccine is the best choice because multiple antigens of the pathogen are needed to induce protective immunity.

Intravacc's outer membrane vesicle pertussis (omvP) vaccine is a promising concept to improve the efficacy and duration of protection against clinical pertussis or whooping cough. A prime/boost vaccination strategy with a stand-alone omvP may strengthen the efficacy of the primary acellular pertussis (acP) vaccination series; omvP could also replace the acP or whole cell pertussis (wcP) component in multivalent vaccines for infants.

#### omvP vaccine concept

The limited duration of protection of acellular pertussis (aP) vaccines compared to whole cell pertussis (wP) vaccines renewed the interest in improved wP and newly developed vaccine concepts like pertussis OMV (omvP).

Similar to wP vaccines, omvP contain relatively large quantities of endotoxin (LOS) and hardly pertussis toxin (PT) or pertactin (Prn), which are known to be highly protective. Moreover, natural vesicle production by *B. pertussis* wild type strains is generally limited.

#### Benefits

- Increased immunogenic properties of antigens in their natural conformation
- Self-adjuvantivity by membrane-bound LOS
- Ability to be taken up by human antigen presenting cells
- Genetic detoxification and improvement of yield and antigen expression as omvP hardly contains intact DNA
- Extended product stability expected
- More protective in mice compared to acP and wcP

Intravacc addresses these challenges by means of genetic engineering:

1. Detoxification of LOS by Lipid A modifications to reduce adverse reactions induced by endotoxin. Intravacc constructed a number of promising Lipid A mutants which are currently under investigation.
2. Enhancing the expression of protective antigens to increase omvP-induced protective immunity against specific antigens. For instance, a Prn.93 mutant in which Prn is retained in the outer membrane and significantly increased omvP induced protection against intranasal infection in mice.
3. Increasing vesicle production by modification of anchor proteins (which bind the outer membrane to the peptidoglycan layer) similar to meningococcal  $\Delta$ mpM-mutant (Waterbeemd, Vaccine, 2010).

## Process development

In production, stress is induced to biomass chemically (e.g. detergent), genetically (modification anchor proteins) and/or physically (e.g. sonification) in order to produce OMV. At Intravacc, we investigate combinations of stress factors to obtain a robust production process with satisfactory OMV productivity and yields.

Intravacc has developed a defined culture medium devoid of animal products, designated as modified THijs medium. This allowed us to improve the upstream processing of pertussis biomass significantly and resulted in a robust, highly reproducible down-scaled production process. Downstream processing of omvP is still ongoing but all non-scalable techniques like centrifugation are already omitted.

## Product characterization

Product characterization and immunoproteomic profiling are valuable tools in the development and the antigenic composition of wP or omvP (by means of mass spectrometry), the reactogenicity (cell stimulation assays) and/or immunogenicity (MIA and 2D-immunoblotting) of intermediate or final products. The quality, safety and efficacy of vaccine concepts can, therefore, be easily and quickly screened before testing the most promising vaccine concepts in animals which saves time and reduces the costs significantly.

## Safety and efficacy

Detergent extracted omvP are well tolerated by mice in the Mouse Weight Gain test and LOS-induced weight retardation was similar to acP vaccine and significantly lower compared to wcP. Detergent-free or naturally formed omvP are currently under investigation for reactogenicity. Preliminary results indicated that non-detergent omvP are more reactogenic, necessitating the genetic detoxification of LOS.

The potency of detergent extracted omvP, aP and wP vaccines was evaluated in the intranasal Mouse Challenge Model. The dose dependent protection of detergent extracted omvP against *B. pertussis* infection was significantly better compared to acP and wcP (Figure 2).

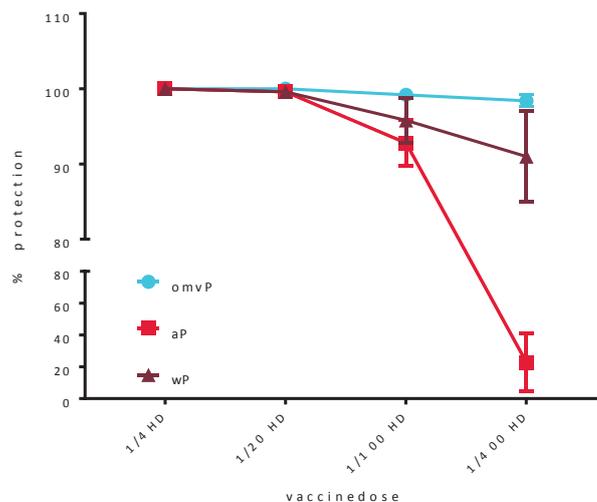


Figure 2: Percentage of protection of vaccinated mice after intranasal challenge with virulent *B. pertussis* in the in-Mouse Challenge Model. Mice were immunized twice (s.c) with 1/4, 1/20, 1/100 and 1/400 human doses (HD) and challenged (i.n) with a non-lethal dose of virulent of *B. pertussis*.

## Intravacc

Intravacc is a renowned, not-for-profit R&D organization. With our unique capabilities and infrastructure, we are able to optimize vaccines, vaccine processes and vaccine technologies. Our aim is to increase equality in access to vaccines throughout the world in order to contribute to public health. We achieve this by transferring our knowledge and technologies to public and private partners worldwide and collaborative R&D. A team of 150 professionals is at your disposal at Utrecht Science Park Bilthoven in The Netherlands

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