Adjuvants: an essential component of *neisseria* vaccines

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Adjuvants may be classified into delivery systems and immune potentiator or modulator molecules based on their mechanism of action. *Neisseria* vaccines containing traditional adjuvants such as aluminium salts have existed for long time, but meningitis caused by *Neisseria meningitidis* serogroups, particularly serogroup B, continues to be a global health problem. Novel strategies have applied *in silico* and recombinant technologies to develop "universal" antigens (e.g. proteins, peptides and plasmid DNA) for vaccines, but these antigens have been shown to be poorly immunogenic even when alum adjuvanted, implying a need for better vaccine design. In this work we review the use of natural, detoxified, or synthetic molecules in combination with antigens to activate the innate immune system and to modulate the adaptive immune responses. In the main, antigenic and imune potentiator signals are delivered using nano-, micro-particles, alum, or emulsions. The importance of interaction between adjuvants and antigens to activate and target dendritic cells, the bridge between the innate and adaptive immune systems, will be discussed. In addition, nasal vaccine strategies based on the development of mucosal adjuvants and *Neisseria* derivatives to eliminate the pathogen at the site of infection provide promising adjuvants effective not only against respiratory pathogens, but also against pathogens responsible for enteric and sexually transmitted diseases.

Keywords: Adjuvant, micro-particle, nano-particle, *Neisseria*, vaccine, delivery system, immune potentiator.

Introduction

Pathogenic *Neisseria* species are mainly responsible for meningococcal and gonorrhoeal disease. Approximately 62 million people get infected annually with gonorrhoea, while *N. meningitidis* is one of the main killers responsible for bacterial meningitis, principally in young children, and the only bacterium capable of generating epidemics taking more than 30, 000 lives each year (1). In this paper, we will focus mainly on the impact of adjuvants on *N. meningitidis* vaccine formulation.

Current meningococcal vaccines are administered by the parenteral route and adsorbed onto aluminium salts; early vaccines used native polysaccharides (Ps) from serogroups A, C, Y, and W_{135} but Ps vaccines are poorly immunogenic in young infants, fail to induce immunological memory and do not provide protection for more than 3-5 years (1). Immunogenicity of Ps was greatly improved when chemically conjugated to a protein carrier, while also inducing long term memory in adults and young infants. Conjugated vaccines have been shown to be very effective, but are too expensive for developing countries, nevertheless adjuvant strategies are being applied to reduce costs and increase immunogenicity. Furthermore, the development of vaccines against serogroup B, accounting for 2000-8000 deaths annually in developed and developing countries, has been vastly hindered because its' Ps is less immunogenic and cross reacts with sialylated proteins in human tissues. Thus, outer membrane vesicles (OMV) containing high amounts of surface protein antigens from the pathogen have been used for epidemic control e.g. in Cuba (2) and Norway (3). Despite the OMV being strain-specific, some level of cross reaction has been detected with the Cuban vaccine (Men B Finlay) (4) Novel strategies using reverse vaccinology (5) and DNA libraries constructed from bacterial genomes (6) have been investigated in an attempt to predict universal antigens to protect against B sero subtypes (7). However, these proteins, peptides or plasmid DNA are also proving to be poorly immunogenic and the traditional alum adjuvant is not sufficient to induce appropriate levels of protection. New adjuvant strategies are therefore being devised based on a combination of these antigens with immune potentiator, molecules and/or delivery systems capable of efficiently targeting immune response components such as dendritic cells (DC).

Immune potentiator, modulator molecules and delivery systems

The innate immune system utilizes multiple receptors (Pattern Recognition Receptors, PRR) of fixed specificity to recognize an enormously diverse array of ligands on microbes known also as Pathogen-Associated Molecular Patterns (PAMPs) (8). The most important PRR studied are the toll-like receptors (TLR) which are transmembrane proteins that recognize

PAMPs like: lipopolysaccharides (LPS, TLR4), lipopeptides (TLR1 and 6), flagellin (TLR5) and nucleic acids (TLR7 or 8, ssRNA; TLR9, unmethylated CpG) from pathogens. To date 13 TLR have been identified in mammals (9).

More than 60 million doses of the Cuban VA-MENGOC-BC® Neisserial vaccine have been administered and it has shown a good safety profile. It is composed of OMV which are nano proteoliposome that contain important porin antigens (PorA and PorB) and native LPS that stimulate DC through TLR4, inducing IL-12 and γIFN cytokines characteristic of a Th1 pattern (10). One of the most important features of neisserial proteoliposomes are their ability to deliver antigenic and immune activating signals to DC (11).

Since LPS has also been described as toxic endotoxin, some groups have worked on detoxified forms of it such as the 3-0-desacyl-4'-monophosphoryl lipid A (MPL) that comes from LPS of the Gram-negative *Salmonella minnesota* R595 or synthetic LPS analogs such as RC529, which are less toxic than native LPS. MPL and RC529 interact with TLR4 inducing a Th1 response similar to native LPS, but have failed to induce long term memory of the stimulated $CD4^+$ T cell subset (12). When these structures have been encapsulated in poly(lactide-co-glycolide) (PLG) microparticles an enhanced immune response has been elicited against *N. meningitidis* B antigens adsorbed on the microparticle surface (13).

MPL adsorbed onto alum is a GlaxoSmithKline Biologicals (GSK) adjuvant used in humans, known as AS04 (14). It has been used with several outer proteins from Neisseria and in addition to the depot effect of alum, co-administered MPL has been shown to redirect the classic Th2 pattern induced by alum alone to a mixed Th1/Th2 response, which favours the induction of protective immune responses. Emulsions have also been formulated with *Neisseria* antigens, including: MF59 a safe oil-in-water adjuvant used in humans (14) and Titermax for experimental use only (15). Lucila et al. (15) demonstrated that formulations using meningococcal C Ps (PsC) conjugated to OMV from *N. meningitidis* B were very efficient in inducing immune responses and long lasting memory in a neonatal mice model. Co-encapsulation of Ps on liposomes with immune potentiator or modulator molecules such as CpG and CD40 is being studied as a non-covalent alternative to conjugated Ps vaccines (16-17) and probably offers a less expensive option to developing countries. OMV have also been used to co-adjuvant the immune response to plasmid DNA (6).

CpG is the ligand for TLR9 and activation leads to an enhanced humoral and cell-mediated immune response through B-cell stimulation to produce more immunoglobulin, as well as promotion of a Th1 pattern and cytokine secretion by DC (18). PLG anionic microparticles uploaded with CpG have been described by Singh et al.(13) as a potent delivery system for co-administered *Neisseria meningitidis* B recombinant proteins and Malyala et al. (19) recently confirmed these results.

Neisseria derivatives as vaccine adjuvants

Neisseria derivatives have been used to adjuvant many antigens and advances in this field of study are perhaps as important as the development of *Neisseria* vaccines themselves. The Adjuvant Finlay PL 1 (AFPL1) is a *Neisseria* derived PL that contains several PAMPs such as LPS, porins, and DNA traces. Ovalbumin incorporated in AFPL1 is very immunogenic when administered by the parenteral route, as are allergen antigens co-adsorbed onto alum with the AFPL1; inducing a Th1 response (20). Alternativly, the mucosally administered Adjuvant Finlay Cochleate 1 (AFCo1) is a microtubular structure derived from *Neisseria* PL interaction with calcium (20). It is more stable and immunogenic than AFPL1 and has also been used to adjuvant parenteral administered antigens from *Leishmania* and malaria (21-22). Intranasal immunization of AFCo1 with incorporated or co-administered ovalbumin has been shown to induce strong systemic and mucosal immune responses (20).

Protollin™ is the commercial name of a proprietary adjuvant from ID Biomedical Corporation (subsequently GSK). This is a non-covalent complex between *Neisseria* proteosomes and *Shigella flexneri* 2ª LPS used mainly as a mucosal adjuvant. Protollin™ is a safe formulation administered to humans inducing mucosal and systemic immune responses against *Shigella* (23) and has also been shown to protect mice from respiratory syncytial virus (24).

Nasal route for *Neisseria* Vaccines

The respiratory tract is the site of entry and colonization of *N. meningitidis*. In many cases non symptomatic individuals can transmit the pathogen to others (25). Parenteral immunization of the current *Neisseria* vaccines is effective in inducing systemic immune responses, however to protect against infection, the induction of immune responses at mucosal surfaces is required (26). Conjugate Ps vaccines induce some level of mucosal immune response and it has been suggested that one of the most important successes of this vaccine relies on the induction of herd immunity through mucosal stimulation (27). Nevertheless, some formulations using liposomes encapsulating serogroup C meningococcal Ps conjugated to *Escherichia coli* heat labile enterotoxin mutant, LTK63 have also been shown to induce potent mucosal and systemic immune response when administered intranasally (28). Similarly, when, the conjugated vaccine Menjugate C was reformulated with chitosan, instead of alum, and intranasally administered to humans, it showed similar systemic immune responses and enhanced mucosal immune responses compared with parenteral administration of the vaccine (29).

OMV from *N. meningitidis* B have also been used in clinical trials; but the nasal immunization required 10 fold more antigen *per* dose than the injectable form to induce similar systemic immune responses. However, these studies did not evaluate mucosal immune responses (30). We too have found that IN immunization with different OMV induce greater mucosal immune responses than parenteral administration (31). The IN route has also been used to test vaccine candidates against *N. gonorrhoea* (32). Recombinant proteins from this pathogen adjuvanted with cholera toxin subunit B induced high immune responses at the genital tract, showing that this route can also be used to stimulate distal mucosal responses.

Commentary

Neisserial vaccine progression is very much related to adjuvant development. Firstly, because *Neisseria* derivatives are being used to adjuvant parenteral and mucosal vaccines candidates from other microorganisms and secondly, novel formulations based on combinations of delivery systems and immune potentiator or modulator molecules are emerging to face the global meningitis problem.

A "universal" B meningococcal vaccine strategy must be accompanied by the selection of the right adjuvants, and enables a number of adjuvant formulations to be examined head-to-head. This would further remove the problem of making the wrong choice of adjuvant or discarding good candidates as a result of selecting a poor adjuvant combination. Conjugated Ps vaccines have represented a huge advance in protecting against *Neisseria* pathogens; however they are too expensive, particularly for the developing world. Current adjuvants could lead to the improvement of new ways to formulate less expensive and equally or more immunogenic antigens as an alternative to conjugated Ps vaccines.

Development of better mucosal adjuvants is another approach to obtain more effective vaccines against *Neisseria,* for the induction of mucosal, as well as systemic immune response, which could potentially protect vaccinees from the pathogen and the population from pathogen spread. We predict that over the next few years, this field will see a plethora of combined current and novel adjuvant technologies directed towards the mucosal route of administration.

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