Mucosal and systemic immune response against Neisseria meningitidis b induced by single time vaccination strategy


Introduction

Vaccination is considered by the World Health Organization (WHO) to be the most cost-effective strategy for controlling infectious diseases but a successful immunization program can contribute much more than just vaccines (1). Epidemiologically targeted implementation of vaccines has diminished morbidity and mortality from many infectious diseases that previously were scourges and economic burdens (such as measles, polio, diphtheria, Haemophilus influenzae type b, meningococcal, and pneumococcal infections) (2). In spite of great successes with these existing vaccines, they are still leading killers, because of their inadequate coverage estimated at: DPT, 89%; TT, 69%; Hep B, 60%; Hib, 69%; Polio, 80%, and Yellow fever, 48%, showing that although these coverages have been increased every year, they are still inefficient (2, 3).

In the case of bacterial meningitis the WHO estimates that 1.2 million cases occur annually and Neisseria meningitidis is the etiological agent in more than 40% of these cases (4).

Several factors have been largely responsible of a difficulty to attain immunization coverage and have been recognized as a problems of current vaccines, such as: the number of dose in the immunization schedule, the excessive use of parenteral route over mucosal route, the inadequate progress in the field of adjuvants for use in human vaccines, higher reactogenicity and unavailability of these vaccines against intracellular pathogens, infected or altered cells such as malaria and HIV, which rely on cell-mediated immunity and scanty feasibility to combined more than one antigen in the same formulation (5).

For meningococcal vaccines, other factors have been added, like the majority of available vaccines protect against some but not all forms of meningitis, and they provide an efficient systemic immune response modify but not eliminate the nasopharyngeal carriage and with this the disease transmissibility.

To bear in mind these principal problems of current vaccines and in light of the ambitious aim of future vaccines, a novel protocol for vaccination named Single Time Vaccination Strategy (SinTimVaS) is proposed. Using female BALB/c mice, we induce systemic and mucosal immune responses against N. meningitidis with only one parenteral and one mucosal dose at the same time, employing the Finlay Adjuvants derivate from N. meningitidis, AFPL1 and AFCo1, respectively. In conclusion, SinTimVas could increase the vaccination coverage and reduce the time-cost of vaccine campaigns, adding the possibility to increase the herd immunity by mucosal specific response induction.

Materials and Methods

AFPL1. Outer membrane vesicles (called proteoliposome or PL when used as antigen and AFPL1, when used as adjuvant)
were produced at industrial scale under GMP conditions at Finlay Institute, Havana, Cuba (6).

**AFCo1.** Adjuvant Finlay Cochleate 1 and the incorporation of non-related antigens were carried out at development scale under GMP conditions at Finlay Institute as previously described (7).

**Animals:** Female BALB/c mice (CENPALAB), 6-8 weeks old and weighing 18-20 g was used to carried out the objectives. **Immunization regiments.** Balb/C mice (n = 3) were distributed in 3 immunized groups and one of control. The first group was immunized with three nasal (IN) dose of AFCo1 (50 µg in 25 µL, 12.5 µL in each nostril) with 7-day interval. The second group was immunized with two intramuscular (IM) doses respectively of AFPL1 (12.5 µg in 50 µL) with 14-day interval. The last group was immunized with one IN dose of AFCo1 (100 µg in 25 µL, 12.5 µL in each nostril) and one IM dose of AFPL1 (25 µg in 50 µL) at the same time.

**Collection of samples.** Saliva and serum were collected to determine anti PL IgA and IgG titres, respectively. Saliva was collected at day 7 after the immunization schedules and serum on day 21 as previously described (8).

**Analysis of immune response.** Anti PL IgA titers in saliva and anti PL IgG, IgG1 / IgG2a in serum were measured by direct ELISA using polystyrene 96-well plates (MaxiSorp F96; Nunc, Roskilde, Denmark) as previously described (8). Anti PL IgG and IgA titres are expressed as unit/mL and arbitrary unit/mL, respectively + standard deviation of the mean from three independent experiments. The anti PL IgG subclasses are expressed in optical density units (OD).

**Statistical analysis.** Significant differences between the means of different groups were determined by a Tukey multiple comparison tests using the Graph Pad Prism 4 Software (CA, USA).

**Results and Discussion**

We have been demonstrated in previous studies, the benefits of use our derivates from *N. meningitidis* B (AFPL1 and AFCo1) in the induction of immune response against this agent (8). Both structures induce an efficient mucosal and systemic immune response with specific IgA response on mucosal secretions by IN rout and specific IgG, IgG1, and IgG2a subclasses responses in serum by all routes, although AFCo1 induces a significant greater mucosal immune response than AFPL1 mainly by mucosal rout (8).

In addition, it has been confirmed for many researches that in general, systemically administered vaccines, although inducing good systemic T cell responses rarely induce optimal mucosal immune responses (9, 10). However others studies had confirmed that parenteral routes can boost the mucosal immune response induced by mucosal vaccination. Many studies combine nasal or/and oral routs with intramuscular routs, using mucosal rout for priming dose and parenteral rout for booster dose (traditional prime-boosts spaced doses) inducing systemic and mucosal immune response (11).

In SinTimVaS using the Finlay Adjuvants and combining mucosal with parenteral rout at the same time, both systemic and mucosal immune responses were induced. With this proprietary new strategy of vaccination (12) against *N. meningitidis* B, one IN dose of AFCo1 simultaneously with one IM dose of AFPL1, induce similar anti PL specific IgG and IgG subclasses antibodies titters in serum than two IM AFPL1 doses or three IN AFCo1 doses, but in addition it induces mucosal specific IgA response (Figure 1).

In conclusion, SinTimVaS induces an efficient systemic immune response as well as mucosal immune response at least in mice.

**Figure 1. Anti PL Immune response induced by SinTimVaS.** Balb/C mice (n = 3) were distributed in 3 immunized groups and one of control. The first group was immunized with three IN dose of AFCo1 (50 µg in 25 µL, 12.5 µL in each nostril) with 7-day interval. The second group was immunized with two IM doses respectively of AFPL1 (12.5 µg in 50 µL) with 14-day interval. The last group was immunized with one IN dose of AFCo1 (100 µg in 25 µL, 12.5 µL in each nostril) and one IM dose of AFPL1 (25 µg in 50 µL) at the same time. Anti PL specific IgG (A), and IgG subclasses (C) antibodies titters in serum and IgA (B) in saliva were measured by direct ELISA. A P-value <0.05 was considered statistically significant.
References