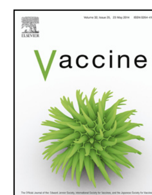
Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Characterization of the immune response induced by pertussis OMVs-based vaccine

Q1 D. Bottero[a,1], M.E. Gaillard[a,1], E. Zurita[a,1], G. Moreno[b,1], D. Sabater Martinez[a], E. Bartel[a], S. Bravo[a], F. Carriquiriborde[a], A. Errea[b], C. Castuma[a], M. Rumbo[b], D. Hozbor[a,*]

[a] Laboratorio VacSal, Instituto de Biotecnología y Biología Molecular (IBBM), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CCT-CONICET La Plata, Calles 50 y 115, 1900 La Plata, Argentina
[b] Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, CCT-CONICET La Plata, 47 y 115, 1900 La Plata, Argentina

ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form 29 February 2016
Accepted 25 April 2016
Available online xxx

Keywords:
Bordetella pertussis
Acellular vaccine
OMVs
Protection
Whooping cough

ABSTRACT

For the development of a third generation of pertussis vaccine that could improve the control of the disease, it was proposed that the immune responses induced by the classic whole cell vaccine (wP) or after infection should be used as a reference point. We have recently identified a vaccine candidate based on outer membrane vesicles (OMVs) derived from the disease etiologic agent that have been shown to be safe and protective in mice model of infection. Here we characterized OMVs-mediated immunity and the safety of our new candidate. We also deepen the knowledge of the induced humoral response contribution in pertussis protection. Regarding the safety of the OMVs based vaccine ($Tdap_{OMVsBp}$,) the *in vitro* whole blood human assay here performed, showed that the low toxicity of OMVs-based vaccine previously detected in mice could be extended to human samples.

Stimulation of splenocytes from immunized mice evidenced the presence of IFN-γ and IL-17-producing cells, indicated that OMVs induces both Th1 and Th17 response. Interestingly $Tdap_{OMVsBp}$-raised antibodies such as those induced by wP and commercial acellular vaccines (aP) which contribute to induce protection against *Bordetella pertussis* infection. As occurs with wP-induced antibodies, the TdapOMVsBp-induced serum antibodies efficiently opsonized *B. pertussis*. All the data here obtained shows that OMVs based vaccine is able to induce Th1/Th17 and Th2 mixed profile with robust humoral response involved in protection, positioning this candidate among the different possibilities to constitute the third generation of anti-pertussis vaccines.

© 2016 Published by Elsevier Ltd.

1. Introduction

Q2

Pertussis is a vaccine preventable respiratory disease that currently needs attention. This disease mainly provoked by *Bordetella pertussis* affects all age groups being infants the most vulnerable population group [1]. Despite the massive use of vaccines for more than 60 years the pathogen represents a major global health problem and one of the top 10 causes of childhood mortality [2]. The first-generation of vaccines still in use in many countries consists of detoxified killed whole bacteria (wP). With its massive use from 1950s the incidence and mortality associated with pertussis fell to very low levels [3]. However, reports on safety concerns in the 1970s shed doubt on the value of wP and contributed to reduce vaccine acceptance in different countries [4–6]. This drawback on wP has prompted the development of a second generation of vaccines, the acellular vaccines (aP) that contain purified proteic antigenic components of *B. pertussis* [6,7]. Although these aP vaccines seemed to keep pertussis under control, during the last two decades the epidemiologic situation of the disease has changed and several outbreaks were reported [8]. Many of these outbreaks were detected in developed countries where wP vaccines composed of a wide range of antigens and used in the primary series were replaced by aP vaccines composed of three to five *B. pertussis* antigens. To explain this outcome, it was speculated that the loss of the vaccine antigen pertactin (PRN) in *B. pertussis* circulating isolates, provides a selective advantage for bacterial survival in aP vaccinated populations [9,10]. Furthermore, the faster waning immunity detected in aP vaccinated population probably due to the Th2 response mainly induced by

* Corresponding author. Tel.: +54 221 425 0497x31.
E-mail addresses: hozbor.daniela@gmail.com, hozbor@biol.unlp.edu.ar (D. Hozbor).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.vaccine.2016.04.079
0264-410X/© 2016 Published by Elsevier Ltd.

UNCORRECTED PROOF

this type of vaccine might also contributes to the increase of pertussis cases. In contrast the wP vaccines and also the natural infection that induce a more long lasting immune response characterized by a mixed Th1/Th17-type CD4+ T-cell response seem to diminish the risk of pertussis [11–16]. The consequences of these differences between the immunity induced by both vaccines have become evident in baboons infection model [17] and humans [18]. This whole scenario points out the need for a third-generation of aP vaccines capable of overcoming the weaknesses associated with the current formulations to improve the disease control. In particular the next-generation aP vaccines should not only induce CD4+ T cells with a mixed Th1/Th17-cell profile, like those induced by infection or immunization with wP vaccine but also must contain more than few purified immunogenic proteins. Following this rationale, our group has recently identified the outer membrane vesicles (OMVs) as a new aP vaccine [19–21]. This vaccine, containing a greater number of immunogens than the current aPs and conformations close to those found in bacteria, behaves safe and highly protective against *B. pertussis* challenge in mouse models. In this study, we investigated with more detail the safety and the immune response induced by OMVs-based vaccine. Comparisons with the commercial aP and wP vaccines currently used are also presented.

2. Materials and methods

2.1. Ethics statement

The studies have been approved by Ethical Committee for Animal Experiments of the Faculty of Science at La Plata National University (approval number 004-06-15 and 003-06-15).

2.2. Bacterial strains and growth conditions

B. pertussis Tohama phase I strain (CIP 8132) was used throughout this study. *B. pertussis* was grown in Bordet–Gengou agar incubated at 36 °C or Stainer–Scholte liquid medium (SS) as previously indicated [22].

2.3. Isolation of outer membrane vesicles (OMVs)

OMVs were produced as previously described [21,23,24]. Briefly, culture samples from the decelerating growth phase were centrifuged and the bacterial pellet obtained was resuspended in 20 mM Tris–HCl, 2 mM EDTA pH 8.5. The suspension was sonicated in cool water for 20 min. After two centrifugations at 10,000 × *g* for 20 min at 4 °C, the supernatant was pelleted at 100,000 × *g* for 2 h at 4 °C. This pellet was resuspended Tris 20 mM pH 7.6. The samples obtained were negatively stained for electron microscope examination [21]. Protein content was estimated by the Bradford method using bovine serum albumin as standard [25].

2.4. Active/pasive immunization and intranasal challenge

Groups of 5 female BALB/c mice (4 weeks of age) mice were immunized systemically with OMVs-based vaccine formulated as previously described [20], commercial wP (Vaksin DTwP) or aP (Adacel™) (1/10 human dose (HD)) using a two-dose schedule over a period of 2 weeks. Two weeks after the second immunization, mice were intranasally challenged with a sublethal dose (10^6–10^8 CFU 40 μl^{-1}) of *B. pertussis* Tohama phase I.

To study protection after by passive transfer experiments, serum (100 μl) from non-immunized mice or mice immunized with two doses of $Tdap_{OMVsBp}$, commercial wP or aP were injected intraperitoneally to BALB/c mice. Twenty-four hours later, the mice were infected with *B. pertussis* Tohama phase I strain (10^6–10^8 CFU 40 μl^{-1}). In all cases, bacterial counts were performed 7 days after the challenge as described previously [19,20]. At least three independent assays were performed in experimental settings.

2.5. Mouse weight gain test (MWG)

The MWG-test was carried out using groups of 8 BALB/c outbred mice (15–20 g) as described [19]. Vaccines were considered non-toxic when passing the WHO and EP requirements [26].

2.6. Stimulation of human whole blood

The whole blood IL-6 cytokine release assay was performed as described by Stoddard et al. [27]. Briefly, a 245 μl of blood sample was dispensed into each well of a 96-well tissue culture plate. Serial dilutions of each vaccine were prepared in RPMI 1640 cell culture medium (GIBCO) in a range of 5–300 ng/ml, incubated with the whole blood and then briefly centrifuged. A sample of 55 μl of plasma from each well was removed and frozen pending quantification of the cytokines. IL-6 levels were measured by ELISA using BD OptiEIA (BD Biosciences) following manufacturer instructions.

2.7. ELISA

Plates (Nunc A/S, Roskilde, Denmark) were coated with sonicated *B. pertussis* Tohama phase I (whole cell lysates) or purified recombinant PTxA both at 3 μg/ml in 0.5 M carbonate buffer, pH 9.5 by overnight incubation at 4 °C. The plates were then blocked with 3% skimmed milk (2 h 37 °C) and incubated with serially diluted mice serum samples (1 h 37 °C). Bound IgG was detected Q3 after 2 h incubation with horseradish peroxidase (HRP)-conjugated goat anti-anti-mouse IgG (Invitrogen, USA). For measuring IgG isotypes, detection of bound antibody was determined using HRP labeled subclass-specific anti-mouse IgG1 (1:8000) or IgG2a (1:1000) (Sigma, Aldrich). Optical densities (OD) were measured with Titertek Multiskan 340 microplate reader (ICN, USA) at 492 nm, and a curve describing the relationship of the OD *vs* the log of the reciprocal of serum dilution was plotted. The inflection point of the curve was determined by GraphPad Prism software. Titers were determined as the reciprocal of serum dilution giving a OD corresponding to the inflection point of the curve.

2.8. Cell line growth

Macrophage-like RAW 264.7 (ATCC TIB-71) were grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) and an antibiotic solution (penicillin 100 units/ml, streptomycin 100 μg/ml) at 37 °C in 5% CO_2.

2.9. Opsonophagocytosis assay

GFP-expressing *B. pertussis* (*B. pertussis* Tohama phase I strain carrying pCW504 kindly provided by Dr. Weiss) were opsonized by incubation at 37 °C with 20% wP-induced/TdapOMVs or aP/naive-induced serum samples for 30 min in a final volume of 40 μl. Serum opsonized GFP-expressing bacteria were incubated with RAW cells at multiplicity of infection (MOI) of 70 for 30 min at 37 °C to allow binding and internalization. After extensive washing to remove non-attached bacteria, samples were analyzed by FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) and phagocytosis was estimated by the mean fluorescence intensity. Results were expressed as mean fluorescence intensity. Statistics analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test (GraphPadPrims®). Differences were considered to be significant when $p < 0.001$.

Please cite this article in press as: Bottero D, et al. Characterization of the immune response induced by pertussis OMVs-based vaccine. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.04.079

Download English Version:

https://daneshyari.com/en/article/10962542

Download Persian Version:

https://daneshyari.com/article/10962542

Daneshyari.com