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EDITORIAL

Outer membrane vesicles: an attractive candidate for pertussis vaccines

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Pertussis is a current public-health problem and major cause of death in children, even in countries with high vaccination coverage [1,2]. This bacterial respiratory disease is caused by *Bordetella pertussis*, but other *Bordetella* species such as *B. parapertussis, B. bronchiseptica*, and *B. holmesii* can provoke symptomatology similar to pertussis [3–5]. The World Health Organization (WHO) estimated that in 2008, about 16 million cases of pertussis occurred worldwide with about 195,000 deaths, 95% in developing countries [6].

For several decades, the infant-immunization programs with antipertussis vaccines had been successful in preventing severe disease. In recent years, however, pertussis has resurged in different countries with a surprisingly high number of cases [7]. For example, in Argentina, the number of pertussis reported cases has increased steadily since 2002, in 2011 quadrupling those detected in 2006 (4.1 vs. 16 per 100,000 inhabitants) [8]. In 2011, 76 deaths were reported in children under 1 year (www.snvs.msal.gov.ar [8]). Another example of resurgence occurred in the US, where the incidence rate in infants with less than 1 year in 2010–2012 duplicated those in 2002 (125 vs. 60 per 100,000 inhabitants). In this country, incidence rates were very high in infants but also in 7- to 10-year-old children and adolescents (13–14 years, http://www.cdc.gov/pertussis/outbreaks/trends.html). Another industrialized country experiencing a notable outbreak is the UK, where in 2012 14 infant deaths were reported (Public Health England; cf. https://www.gov.uk/government/publications/whooping-cough-pertussis-statistics, accessed October 2016).

This epidemic in different countries has moved the scientific community and health professionals to seek an understanding of this alarming new situation, to identify the causes [9–11] and review and implement new strategies for the control of pertussis [12]. Several factors apparently contribute to this pertussis-case increase, probably some occurring at a different weight depending on the country and the population considered. Nevertheless, a consensus exists in identifying, as part of the causes of the epidemic, several factors related to the vaccines currently in use and the vaccination—e.g. suboptimal coverage of the three primary doses, noncompliance with vaccination-schedule timing (delayed vaccination) [13,14], the waning of vaccination-conferred immunity [15–17], and the circulation of a resistant bacterial causative-agent population resulting from the selection pressure exerted by mass vaccination [11].

Currently, two types of vaccines against pertussis are in use: the whole-cell vaccines (wP) constituted by a suspension of detoxified heat-killed bacteria and acellular vaccines (aP) consisting of purified *B. pertussis* immunogens. wP was the first developed against the disease. With the massive use of this vaccine in the 1950s, the incidence and mortality associated with pertussis fell to very low levels. Reports on safety concerns in the 1970s, however, cast doubt on the wP vaccines' value since they were associated not only with side effects at the injection site but also serious systemic reactions [18,19]. These drawbacks and, to a lesser extent, the low effectiveness of particular wP vaccines contributed to reducing pertussis-vaccine acceptance in countries such as the UK, Italy, Ireland, Australia, West Germany, Russia, Japan and Sweden [19,20].

The widespread apprehension about wP prompted the development of acellular vaccines containing purified antigenic-protein components of *B. pertussis* (2, 3, or 5 immunogens) [21,22].

Though finally there is no evidence to suggest that wP vaccines cause severe adverse reactions such as brain damage or severe neurological disorder, the aP vaccines are more accepted, especially in industrialized countries where they have gradually superseded the wP formulations. Currently, most of the countries of the EU and US use only aP vaccines. The aP formulations restored people's confidence in *pertussis*-containing vaccines, and infection appeared controlled for several years. Notwithstanding, during the last two decades, the epidemiology of pertussis has changed [7,23] with several major outbreaks occurring, the incidence of which not only indicated a waning immunity but also demonstrated that the wP vaccines gave children a more lasting immunity than aP [24–26]. Furthermore, the risk of pertussis was increased in school children and adolescents vaccinated exclusively with aP compared to those receiving only one wP dose [26,27]. This difference could result from the weaker immune response induced by aP vaccines characterized mainly by Th2 profiles [28]. In 2015, the Strategic Advisory Group of Experts on immunization expressed concern regarding the resurgence of pertussis in certain industrialized countries despite high aP-vaccine coverage [29]. The switch from wP to aP for primary

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infant immunization was proposed as at least partially responsible for that resurgence. WHO therefore recommended that the switch be considered only if, in the national immunization schedules, large numbers of doses including several boosters can be assured. Countries currently using aP vaccines may continue using them, but should consider the need for additional booster doses and strategies to prevent early-childhood mortality upon pertussis resurgence.

Within this context, along with optimizing the use of current vaccines (i.e. vaccination during pregnancy, cocooning strategy, etc.), a need to develop new and improved vaccines is urgent. Because no absolute correlate for protection exists, the task is therefore difficult. Data from animal models and human studies, however, indicate that although antibodies may mediate protection, Th1 and Th17 cellular responses are responsible for long-lasting protection [28]. To induce or drive a Th1 and Th17 response, different approaches have already been proposed [30–32]. Here, I will describe the vaccine candidate designed by us.

1. Bases for designing our novel pertussis vaccine

Component vaccines, or subunit vaccines, are particularly attractive from the standpoint of reduced toxicity, since they are safer than vaccines constituted by the entire organism, either dead or attenuated [33]. Despite the advantages in safety, a fundamental limitation is their inability to stimulate a strong immune response *in vivo* when administered alone. Therefore, to enhance their immunogenicity, they should be combined with adjuvants, conjugated to polysaccharides, or formulated in antigen-controlled-release systems [34,35]. Controlled-release technologies have become promising strategies for protein-immunogen presentation and release, as the protein immunogens present in these delivery systems have similar conformations to those of the pathogens; moreover, they are more readily internalized by antigen-presenting cells. Some of these controlled-release technologies, however, require purification steps and the antigen encapsulation that normally make these approaches costly, especially in developing countries.

A promising option for replacing the above-mentioned strategies is the use of outer-membrane vesicles (OMVs) that naturally contain bacterial surface antigens. Indeed, two meningitis vaccines containing components derived from the outer membrane and periplasm of *Neisseria meningitidis* serogroup B are currently available [36,37]. Though OMV-containing meningococcal vaccine has been approved by regulatory agencies, recent work on meningitis B vaccines shows that OMVs can be modified to yield an OMV product that is safer and effective [38]. Data on the safety and efficacy of these vaccines and the knowledge that most Gram-negative bacteria secrete vesicles naturally make vesicle-vaccination strategy feasible for other diseases [39]. Within this context, we designed a new pertussis vaccine based on OMVs derived from *B. pertussis*, which can be used as a combined vaccine with at least tetanus and diphtheria toxoids. The OMVs derived from *B. pertussis* range in size from approximately 50 to 200 nm in diameter and enclose many native bacterial antigens within the spherical particles. We characterized the composition of the pertussis nanoparticles at >200 protein components—including the virulence factors pertussis toxin, pertactin, fimbriae, filamentous haemgglutinin, and adenylate-cyclase [40,41]. The mean content of the main *B. pertussis* protective immunogen, the PTx in OMVs is 15.1 ± 3.2 ng/μg of total OMVs proteins. The biotechnological process used for obtaining the OMVs contains steps of sonication, centrifugation, and tangential cross-flow filtration. This process is robust and reproducible. To date, we have obtained over 40 batches of *B. pertussis*–derived OMVs. Similar morphology, size distribution, and presence of surface immunogens identified first by two-dimensional electrophoresis associated to matrix-assisted laser desorption ionization-time-of-flight mass spectrometry analysis and then by orbitrap technology were observed in all the obtained batches [40]. It is interesting to note that the presence of a high number of immunogens in the vaccine formulation is essential since they may avoid the sufficient selective pressure conferred by a single or a few protective-vaccine antigens.

To characterize functionally the OMV-based vaccine, we formulated the OMVs with tetanus and diphteria toxoids [41,42]. The safety of this vaccine was evaluated by a mouse weight-gain test and the murine and human whole-blood IL-6-release assays. The latter assay that evaluates the levels of IL-6 as indicator of proinflammatory response was described previously for *Neisseria* vaccines [43]. The OMV vaccine prepared by us fulfilled the WHO criteria for safety in the weight-gain test. Results from mouse and human whole-blood assays also demonstrated that the wP exhibits a higher endotoxic activity than the commercial aP- and OMV-based vaccines [44]. In agreement with these results, we determined that the lipopolysaccharide content in the OMV-based vaccines is at least 50 times lower than the commercial wP.

The protection capability of the OMV-based vaccine was evaluated by the mouse-intranasal-challenge model [41,42,45]. Using this model, we detected that OMV-based vaccines are effective against strains expressing vaccine/reference type *ptx*P1, *ptx*A2, *prn*1, *fim*3-1 (Bp Tohama phase I) and *ptx*P4, *ptx*A4, *prn*6, *fim*3-1 (Bp18323), and non-vaccine-type alleles, *ptx*P3, *ptx*A1, *prn*2, *fim*3-2 (circulating strain Bp106) [42], including pertactin-negative isolates whose prevalence has increased in recent years in countries that only use aP in their calendars [46]. In contrast, commercial aP vaccine used in a dose in which PTx content was equivalent to OMV-based vaccine, showed little protection effect against all genotypes tested. wP vaccine used in 1/20 of human dose also exhibited lower protective capacity against *ptx*P3, *ptx*A1, *prn*2, and fim3-2 (circulating strain Bp106).

As wP, our vaccine candidate induced a mixed Th1-Th17-Th 2 profile and conferred a long-term protection in the murine model [42,44]. In contrast, commercial aP administered with alum as the adjuvant induced Th2 and Th17 cells, but weak Th1 responses. The long-term protection for the meningococcal vaccine based on OMVs is currently under discussion. It was reported that the duration of antibody titers induced by the commercial Bexsero vaccine appeared shorter than that following MenC conjugate vaccination [47]. This seems not the

case for OMVs pertussis vaccine since memory response seems to be associated with such mixed Th1-Th17-Th2 profile induced. We recently detected that the transfer of spleen cells from mice immunized with our OMV-based–vaccine-induced protection in recipient mice (manuscript in preparation). Moreover, OMV vaccination also induced, as did wP, a robust antibody response (total IgG titer: 4710.00 ± 723.91 for wP and 1387.41 ± 221.67 for OMV-based vaccine) with a high IgG2a/IgG1 ratio. OMV-induced antibodies also transferred protection against *B. pertussis* infection. Antibodies may confer protection by several mechanisms. We observed that *B. pertussis* was efficiently opsonized by OMV-induced antibodies. The antibody responses induced by OMVs were directed against GroEL, outer-membrane-protein complex but also pertussis toxin, fimbriae, and pertactin [44].

OMVs could also be used in combination with commercial aP vaccines to direct their immune response to a Th1 profile without affecting the protective capacity of both vaccines. We also obtained excellent protection against *B. parapertussis* [48] and *B bronchiseptica* with OMVs derived from those pathogens (manuscript in preparation).

In summary, our formulation based on OMVs derived from *B. pertussis* contains a greater number of immunogens and conformations close to those found in the causal agents than the commercial aP vaccines. Moreover, OMVs are 10 times smaller in diameter than whole bacteria, thus possibly increasing the exposure to different cell types. OMVs may furthermore reach immune cells deeper in the tissues that are less accessible for the whole bacteria or large alum particles to induce an immune response [49]. Our vaccine formulation is less toxic than wP and likewise attractive from an economic standpoint. The final cost per dose would be lower than that of the existing acellular vaccines based on several purified protein immunogens one by one—a detail clearly impacting the final cost of the vaccine. With our OMVs, a single procedure is necessary for their production. Furthermore, our OMV platforms evidence a promising protection spectrum for developing new vaccines against other *Bordetella* species [48].

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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