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Development of improved pertussis vaccine

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Rates of infection with *Bordetella pertussis*, the gram-negative bacterium that causes the respiratory disease called whooping cough or pertussis, have not abated and 16 million cases with almost 200,000 deaths are estimated by the WHO to have occurred worldwide in 2008. Despite relatively high vaccination rates, the disease has come back in recent years to afflict people in numbers not seen since the pre-vaccine days. Indeed, pertussis is now recognized as a frequent infection not only in newborn and infants but also in adults. The disease symptoms also can be induced by the non-vaccine-preventable infection with the close species *B. parapertussis* for which an increasing number of cases have been reported. The epidemiologic situation and current knowledge of the limitations of pertussis vaccine point out the need to design improved vaccines. Several alternative approaches and their challenges are summarized.

Keywords: whooping cough, pertussis, *Bordetella pertussis, Bordetella parapertussis*, vaccines

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Pertussis or whooping cough is a highly contagious vaccine-preventable respiratory disease. All age groups are susceptible to pertussis infection; however the disease is more severe in unvaccinated infants in whom potentially fatal complications such as convulsions, bronchopneumonia and encephalopathy can occur. The gram-negative bacteria *B. pertussis* is the main disease-causing agent. However, the closely related species *B. parapertussis* also can induce these symptoms, as has been reported in increasing numbers.[1,2]

For several decades, infant immunization programs with pertussis vaccines have been very successful in preventing severe disease.[3,4] However, in recent years the disease has reemerged in many communities/states/countries with a surprisingly high number of cases.[5-9] The global pertussis burden estimated in 2008 by the WHO is ~16 million cases and 195,000 deaths per year, with the highest incidence rates and major risk of deaths and complications occurring in infants.[10] In Argentina, the number of reported cases has increased steadily since 2002. The number of cases reported in 2011 quadrupled from those reported in 2008.[11] In 2011, 76 deaths were reported in < 1-y-old children (www.snvs.msal.gov.ar). It is noteworthy that in developed countries like the US or Australia, the number of cases reported in 2010–2 at least tripled compared with those from a few years earlier.[9,12-15]

This alarming situation has moved the scientific community and health professionals to seek an understanding of this new situation. Several factors that could explain the resurgence of the disease have been proposed, most of them associated with current vaccines: waning vaccine-induced immunity, the switch from whole cell vaccines (wP) to acellular vaccines (aP) and pathogen adaptation.[16] The wP vaccines were developed and used since the 1950s in western countries, reaching almost worldwide application in the 1970s. The aP vaccines were developed because of concerns raised by reports of neurological and other adverse reactions in children upon wP administration. Because of such concerns and the lower rate of adverse effects caused by the aP vaccination compared with wP vaccines were gradually replaced in the 1980s and 1990s by aP vaccines in some developed

countries.[17] Beside the use of these vaccines, outbreaks of pertussis also were observed among vaccinated individuals within just a few years of vaccination.[18] This suggested that immunity from vaccination waned and that protection against the disease and infection was incomplete. In order to reinforce the defenses against the pathogen, in the last years several boosters have been included in national vaccination programs, including a universal booster dose with aP vaccine for preadolescents and a booster dose for pregnant women.[19,20]

The switch from wP to aP vaccines seems to have complicated the pertussis situation because immune responses elicited by aP vaccines are less robust. In a non-human model it was shown that aP vaccines protect against disease but fail to prevent infection and transmission.[21] A case-control clinical study designed to assess the risk of pertussis among 10- to 17-y olds during the 2010–1 outbreak in California revealed that teenagers who had received four wP doses were nearly six times less likely to have been given a diagnosis of pertussis than those who had received all aP vaccines and nearly four times less likely than those who had received a mix of vaccines.[22] Another study also found that the risk of pertussis was increased in schoolchildren and adolescents whose infant schedule was composed exclusively of aP doses compared with subjects who received ≥ 1 wP dose.[23,24] These findings are consistent with the knowledge that aP vaccines elicit a mixed Th2/Th17 response which is less effective than the strong Th1 response induced by natural infection or wP.[25] This observation has been confirmed in a baboon model.[26]

In addition to waning of vaccine-induced immunity, changes in the antigenic and genotypic characteristics of circulating *B. pertussis* strains are being described: alleles of vaccine antigens expressed by circulating bacteria largely differ from those expressed by the strains used in vaccine production.[27] The emergence of these predominant strains seems to reflect selective pressure from vaccination. In some but not all countries, the emergence of allelic variants coincides with disease resurgence. More recently, strains have emerged that do not express one or more components of pertussis vaccines, in particular pertactin.[28,29] It was reported in the US that pertactin-deficient isolates increased by > 50% in 2012.[30] Similarly in Australia where a large outbreak of pertussis occurred during 2008–12, it was detected that 30% (96/320) of *B. pertussis* isolates did not express pertactin.[31] Observations that pertactin-deficient isolates were only detected in regions where aP vaccines were the only vaccine used suggest that aP vaccination resulted in the expansion of strains that have a selective advantage in vaccinated human populations.

Other factors that also contributes to pertussis resurgence is the increased number of cases caused by *B. parapertussis* for which no available vaccines confer protection.[32,33] Little attention has been paid to this bacterium because it causes a milder illness than *B. pertussis* and the rate of detection seems to be low. However, recent studies have revealed high rates of detection in patients with whooping cough in some field studies. Watanabe et al. revised the current literature, pointing out that it is necessary to pay greater attention to infections caused by this bacterium.[33] To this aim it is important to improve the specific diagnosis test for B. *parapertussis.*

Under this entire context it is clear that a new generation of vaccines capable of overcoming the weaknesses associated with the current vaccines is needed to improve disease control. Several pathways deserve to be explored even without there being a universal solution, since each country has a different situation depending on the history of vaccination policy developed in the last decades. One possibility is to promote the use of wP vaccines but with new less reactogenic[34] and improved[35] formulations. However, the return to wP vaccine use would be difficult for the public to accept in countries that switched to aP vaccines due to adverse reactions from wP vaccines.

Another possibility is to improve aP vaccine by the inclusion of additional virulence factors of *B. pertussis* such as adenylate cyclase toxin or iron regulated proteins[36,37] and/or an adjuvant able to drive a high Th1 response, i.e., Toll-like receptor agonists, as has been discussed recently.[38]

There are several new vaccine candidates that may contribute to solving the problem of the reemergence of pertussis. The use of an attenuated *B. pertussis* strain administered intranasally has been successfully tested in animal models and moved to Phase I clinical trials.[39,40] Although this is a promising application that has shown some advantages,[41,42] it shares the potential safety concerns related to the use of attenuated pathogens.[39]

An interesting alternative that combines some of the above-mentioned strategies is the use of outer membrane vesicles, also called nanoparticles, which contain bacterial surface antigens. There are two meningitis vaccines containing components derived from the outer membrane and periplasm of *Neisseria meningitides* serogroup B.[43] Data on safety and efficacy of these vaccines and the knowledge that the majority of Gram-negative bacteria secrete outer membrane vesicles may make vaccination strategy with outer nenbrane vesicles (or nanoparticles) feasible for other diseases.[44] In this context, we designed an aP nanoparticle vaccine derived from *B. pertussis* and *B. parapertussis*, with protective effect in the accepted model of intranasal challenge in mice against different genetic background strains of *B. pertussis* and *B. parapertussis*.[45-47] This vaccine formulation has a safety profile in mice that is comparable to that of commercial aP and much better than wP. Furthermore, it elicits a protective immune response with a mixed Th1/Th2 profile and induces also a robust antibody response. We have characterized the composition of the pertussis nanoparticles, finding > 40 protein components, mostly membrane-bound proteins. The presence of a high number of immunogens in the vaccine formulation is important since this may avoid the excessive selective pressure conferred by a single or a few protective vaccine antigens.[48] This formulation also is attractive economically, which is critical for its use in developing countries. It is estimated that the final cost per dose is less than that of existing aP formulations based on several purified protein immunogens, which impacts on the final cost of the vaccine. In our case

a single process step is necessary for the production of each *B. pertussis* and *B. parapertussis* nanoparticle.

In summary, evidence that the current aP vaccines are not providing optimal control of pertussis has enhanced the interest in improvements of current vaccines and in the development of new vaccines. Several options such as inclusion of new antigens or adjuvanting the current aP vaccine to change the Th profile are under debate. On the side of new vaccines, a live attenuated strain is a potential option. In this category, the formulation based on nanoparticles (outer membrane vesicles) of *B. pertussis* and *B. parapertussis* seems to be a good alternative to current formulations containing a greater number of immunogens that current aP vaccines and conformations close to those found in bacteria, favoring protective capacity. These formulations also provide protection against *B. pertussis* and *B. parapertussis,* extending the range of protection over current vaccines, which would contribute to better disease control. Tese different options are not mutually exclusive and may adapt differently to situations that are not similar due to the previous history of vaccination in the different countries.

Disclosure of Potential Conflicts of Interest

The Author states he has no conflict of interest

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