New approaches in pertussis vaccines for developing countries

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The currently available pertussis vaccines are highly efficacious. However, whole cell vaccines display some reactogenicity and acellular vaccines are less toxic, but elevated costs limit their use in developing countries. Instituto Butantan, Brasil, has developed a new technology to remove lipopolysaccharides from the whole cell vaccine using a simplified and not expensive methodology, which also provides monophosphoryl lipid A as by-product, to be used as adjuvant. This new whole cell pertussis vaccine with low LPS content combined with diphtheria and tetanus antigens (DTP_{low}), was shown to be as potent as the regular DTP vaccine and less reactogenic. As another by-product, an alternative acellular pertussis vaccine can be produced by a simple low cost procedure. A recombinant BCG-Pertussis vaccine was developed which can be given at birth as a single dose, potentially covering the neonate susceptibility before immunity conferred by pertussis vaccination schedule becomes effective.

Keywords *Bordetella pertussis*; whole cell pertussis vaccine; acellular pertussis vaccine; monophosphoryl lipid A, recombinant BCG

1. Introduction

The early developments of simple and low cost vaccines, stimulated the emergence of Pasteur Institute-like vaccine producers, most of which vanished under the pressure of Good Manufacturing Practices (GMP - Quality System Regulation) on the development of new vaccines. Experience has shown, both for public and private small producers, that unless they are linked to research and development, simple technology transfer will not keep them very long in operation. However, high cost international R&D centers have not fulfilled the needs.

Whooping Cough, also known as pertussis or "long cough", is an infectious disease of the upper respiratory tract, caused by *Bordetella pertussis*. According to the World Health Organization, this disease attacks about 60 million children every year, being responsible for 600.000 deaths, mainly in underdeveloped or developing countries, with precarious sanitary conditions or lack of vaccination programs. Infants, particularly those under six months of age continue to be the more susceptible group in the disease burden and mortality [1]. Although pertussis has been the most commonly reported vaccine-preventable disease among children under 5 years of age, it is the only one with an increasing reported incidence [2].

The whole cell pertussis vaccine (WCPV) was described in the decade of 1940, by Kendrick and collaborators [3]. The WCPV, as a component of the diphtheria-pertussis-tetanus toxoid (DPT) vaccine, has been shown to be very effective in protecting humans against *Bordetella pertussis* infection [4, 5]. It is prepared with bacterial cells obtained by centrifugation or filtration of the culture in liquid media, submitted to inactivation by formaldehyde, thimerosal or thermal treatment. The immunization with WCPV has been used worldwide, with high efficiency in the control of the disease. In spite of being very efficient with high coverage, the detoxification step in the vaccine production is one of the crucial points of the process, with undesirable adverse events being described [6]. This has led to resistance against vaccination campaigns, and some countries interrupted pertussis immunization, resulting in outbursts of pertussis infection [7]. This stimulated the development of new acellular pertussis vaccines (APVs),

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composed by the main antigenic elements of the bacteria [5, 8, 9]. Several comparative studies on phase II efficacy trials have demonstrated that acellular pertussis vaccines are efficient and less reactogenic than the whole-cell DPT vaccine [8, 9]. Most of these preparations differ between themselves qualitatively and/or quantitatively as to the antigenic components used. Whichever the formulation chosen by the producer, the cost of the product is high, since several chromatographic steps of purification of the supernatants or filtrates of culture are usually required, in order to obtain each one of the antigen components of the vaccine. Although the whole-cell DTP vaccine is no longer used in developed countries, the high production costs of the acellular vaccines limits their widespread use in developing countries, and therefore the WCPV is still purchased by the World Health Organization and distributed to developing nations because of its much lower cost.

2. Low cost procedures in the development of new pertussis vaccines

2.1 Whole cell pertussis vaccine with low rates of LPS and MPLA as by-product

Instituto Butantan has recently developed a simple procedure to remove bacterial lipopolissacharide (LPS) from *Bordetella pertussis*, producing a non toxic and safer whole cell pertussis vaccine, with low LPS content (WCP_{low}). The new vaccine is obtained from culture cells of *B. pertussis*, detoxified by formaldehyde and extracted with organic solvent [10]. This product was approved by the Mouse Weight Gain test (MWGT) for assessment of specific toxicity [11] and the Mouse Protection test (MPT) [3] to estimate the potency, the required in vivo assays for the release of Whole Cell Pertussis Vaccine. This procedure also yields a significant amount of monophosphoryl-lipid A (MPLA), which has been used as adjuvant in pre-clinical tests of the Butantan APV (see bellow) and other vaccines. The WCP_{low} vaccine was shown to be as potent as the traditional WCPV produced by Instituto Butantan, with a 5 to 10 times lower content of pyrogen. The WCP_{low} is currently being tested in a Phase I clinical trial in Brazilian children, combined with tetanus and diphtheria toxoids. In the next steps of the field trial, the vaccine will be evaluated as combined quadruple preparations (diphtheria, pertussis, tetanus and haemophylus or diphtheria, pertussis, tetanus and hepatitis B) or as a pentavalent composition (diphtheria, pertussis, tetanus, hepatitis B and haemophylus) vaccine. The technical simplicity of the process doesn't result in a rise in price nor are the technologies more complicated, as compared with those already standardized for production of the traditional pertussis vaccine. The production of the WCP_{low} vaccine against whooping cough, less toxic and at a cost of about US$ 0.13 per dose for the diphtheria-tetanus-pertussis combined vaccine (DTP_{low}), certainly will be of great impact to the Public Health System.

2.2 Acellular pertussis vaccine at a low price

In the process of obtaining the whole cell pertussis vaccine, the culture filtrate, is a disposable product that contains the raw material for the production of an acellular pertussis vaccine (APV) [12], including immunogenic antigens secreted during growth of the bacteria. The system can provide about 5% of an alternative APV, required for a small proportion of children with neurological problems or strong adverse reactions to the WCPV. The process consists of the molecular ultrafiltration of the culture filtrate, previously detoxified in formaldehyde, in 30KDa membrane (Millipore), followed by a sterilizing filtration in 0.22 μm membrane. This novel APV has been proven to be safe, immunogenic and highly protective in mice, in the intracerebral pertussis protection assay [3], as a quadruple vaccine, combined with diphtheria and tetanus toxoids and hepatites B vaccine (Fig. 1).

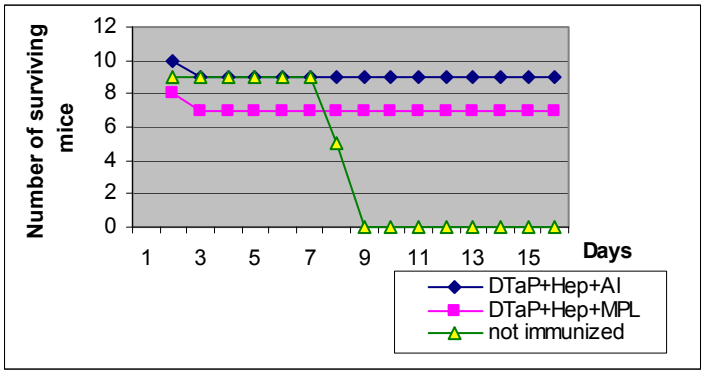

Fig. 1. Survival of mice immunized with the combined diphtheria-tetanus-acellular pertussis- (DTPa) plus Hepatites B (Hep) vaccine after challenge using aluminium hidroxyde (Al) or monophosphoril-lipide A (MPL) as adjuvants. Female mice (14-16g) were immunized with the vaccine, boosted after 2 weeks and challenged 15 days after the booster with a virulent *B. pertussis* strain [3].

The process as a whole, including the production of the two pertussis vaccines, WCP_{low} and APV, and an adjuvant, MPLA, is obtained as an "in line" production process, without rise in the final price of the traditional WCPV.

2.3 Recombinant BCG-Pertussis vaccine

Natural immunity or that acquired by vaccination are not life-long induced and protection is very low after 10 years without boosting [13, 14, 15], which results in an increasing number of adults and adolescents with waning immunity and susceptibility to infection. These groups are a source of infection for young infants with not-yet-complete primary vaccination schedules. Although acellular vaccines are a significant improvement on DPT vaccination, they still require multiple doses to achieve maximum efficiency. The development of a low-cost pertussis vaccine that immunizes efficiently with only one dose would be particularly important for developing countries, where difficult access to health centers hinders attaining complete vaccination of children.

Institute Butantan is developing a recombinant BCG-Pertussis vaccine that will allow the start of pertussis immunization in the first week of life, before the standard vaccine calendar. *Mycobacterium bovis* BCG is considered a high-potential candidate as host for the presentation of heterologous antigens in the development of new live recombinant vaccines [16, 17]. A multivalent vaccine based on recombinant BCG (rBCG) would have several advantages: (i) one dose would be sufficient to confer long-lasting cellular immunity; (ii) the World Health Organization recommends that BCG be administered at birth; (iii) it is the best-known adjuvant in animals and humans; and (iv), it has low production costs and is thermostable. A pertussis vaccine based on rBCG could improve general immunization, since BCG can be administered at birth and requires only one dose to induce long-lasting immunity. Most developing countries use BCG for immunization against tuberculosis. rBCG expressing pertussis antigens could induce protective immunity against *B. pertussis* infection. Pertussis toxin (PT) is the most important antigen characterized so far for *B. pertussis* and is a component of all approved and commercially available acellular vaccines [8, 9, 10]. It is composed of five subunits; S1 is the active domain, and S2 to S5 arrange to form the binding domain [18]. A genetically modified version of S1 subunit has been used to produce a *B. pertussis* whole-cell vaccine containing nontoxic PT or to compose acellular DTP (DTaP) vaccines [19]. We have constructed a rBCG strain expressing the genetically detoxified S1 subunit of pertussis toxin. 9K/129G (S1PT), in fusion with the β-lactamase signal sequence, under control of the upregulated *M.* β-lactamase promoter, pBlaF*[20], and the antigen was localized to the mycobacterial cell wall. The expression vectors were relatively stable in vivo, since at

two months, 85% of the BCG recovered from the spleens of vaccinated mice maintained kanamycin resistance.

The rBCG-S1PT strains induced a very low humoral response against PT. On the other hand, spleen cells from rBCG-S1PT-immunized mice showed elevated gamma interferon (IFN-g) and low interleukin-4 (IL4) production, as well as increased proliferation, upon pertussis toxin (PT) stimulation, characterizing a strong antigen-specific Th1-dominant cellular response. Mice immunized with rBCG-S1PT strains displayed high-level protection against an intracerebral challenge with live *Bordetella pertussis* (Table I), which correlated with the induction of a PT-specific cellular immune response, reinforcing the importance of cell-mediated immunity in the protection against *B. pertussis* infection.

Table I – Intracerebral Challenge of rBCG-Pertussis immunized mice with live ***B. pertussis***[a]

Group	No of surving/ Total of mice	Day of death after challenge
Saline	1/8	5, 6, 9, 12,12,12,12>13
rBCG-Pertussis	7/9	6, 8, >12, >12, >12,>12>12>12, >12
DPT	8/9	12, 13, >13, >13, >13, >13, >13, >13

[a] Groups of female Swiss mice, previously vaccinated with 10^6 CFU/0.5 ml of the BCG or rBCG strains expressing S1PT, or the saline and DPT controls, were challenged i.c. with 18,500 CFU of live *B. pertussis* in 30 µl saline, and survival followed for 13 days.

Our results suggest that rBCG-expressing pertussis antigens could constitute an effective, low-cost combined vaccine against tuberculosis and pertussis. Since neonatal *Mycobacterium bovis* BCG vaccination against tuberculosis is used in most developing countries, a live recombinant BCG-pertussis vaccine could induce protection in susceptible infants and young children before immunity by the pertussis vaccination schedule becomes effective.

These appropriate technology developments shared with developing country vaccine producers could support and improve their contribution to public health, by providing locally made vaccines and by fostering new investigations.

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