Title: Immunogenicity of whole cell low lipopolysaccharide pertussis vaccine in infants

Running Title: Immunity to whole cell low LPS pertussis vaccine in infants

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Abstract

The lack of a clear correlation between the levels of antibody to pertussis antigens and protection against disease lends credence to the possibility that cell-mediated immunity (CMI) provides primary protection against disease.

This phase I comparative trial had the aim of comparing the in vitro cellular immune response and anti-pertussis toxin IgG titers (anti – PT) evaluation of a cellular pertussis vaccine with low lipopolysaccharide (LPS) content (wP_low) with that of the conventional whole-cell one (wP). A total of 234 infants were vaccinated at 2, 4, and 6 months with conventional wP or wP_low. Proliferation of CD3+ T cells was evaluated by flow cytometry after 6 days of peripheral blood mononuclear cells culture, with heat-killed B. pertussis or phytohemagglutinin (PHA) stimulation. CD3+, CD4+, CD8+ and TCR γδ+ cells were identified in the gate of blast lymphocytes. IFN-γ, TNF-α, IL-4 and IL-10 levels in supernatants and serum anti-PT IgG levels were determined using enzyme–linked immunosorbent assay (ELISA). Net percent CD3+ blasts in cultures with B. pertussis was higher in the group vaccinated with wP (medians of 6.2% for wP and 3.9% for wP_low; p=0.029). The frequency of proliferating CD4+, CD8+ and γδ+ cells, cytokine concentrations in supernatants and the geometric mean titers of anti-PT IgG were similar between vaccination groups. There was a significant difference between the T cell subpopulations for B. pertussis and PHA cultures, with a higher percentage of TCR γδ+ cells in those of B. pertussis (p<0.001). The overall data did suggest that wP resulted in modestly better specific CD3+ cell proliferation and TCR γδ+ cells expansion was similar for both vaccines.

Keywords: Pertussis vaccine; Lipopolysaccharide; Cellular immunity, humoral immunity, infant.
Introduction

Efforts to immunize against whooping cough began almost as soon as Bordet discovered the causative bacteria, *Bordetella pertussis*, in 1912 (8). Whole-cell pertussis (wP) vaccine has been available since 1940. Yet the road to eliminate circulation of the pathogen proved arduous. Throughout the world, pertussis remains a major cause of morbidity and mortality among infants (45). Some 20-40 million cases of disease occur worldwide each year, 90% of which are found in developing countries (44).

Although no causal link has been identified between wP vaccination and permanent brain damage or death, concerns about systemic reactions after immunization with wP vaccine have been a major factor in its reduced acceptance in developed countries (46). This has led to the development of more purified and less locally reactogenic acellular pertussis (aP) vaccines (21, 22, 43, 47). Similar immunogenicity is obtained with both types of vaccine, as the most efficacious vaccines of either category protect >80% of the recipients from clinical disease. Moreover, the considerably higher development costs of aP result in prices per dose that are unlikely to be currently affordable for developing countries (46).

Lipopolysaccharide (LPS) possesses endotoxic activity and has also powerful adjuvant activity. Both these properties are based upon the recognition of the LPS by the host Toll-like receptor complex TLR4 / MD-2 and the subsequent activation of NF-κB (35). The relatively high reactogenicity of wP vaccines has been associated with pro-inflammatory cytokines (2, 28). Hence, a straightforward approach to reduce wP reactogenicity would be the generation of a pertussis vaccine with a reduced quantity of LPS. Furthermore, the lack of a clear correlation between the levels of antibody to pertussis antigens and protection against disease, the persistence of protective immunity long after the disappearance of pertussis antigen-specific antibody (Ab), and the longer
duration of T cell responses to pertussis antigens lend credence to the possibility that cell-medicated immunity (CMI) provides primary protection against disease (16, 34).

This phase I study was then performed to obtain preliminary immunogenicity data of a new cellular pertussis vaccine with low LPS content (wP\textsubscript{low}), in comparison to the conventional whole-cell pertussis vaccine (wP) used in Brazil, all of which formulated with diphtheria and tetanus toxoids and given in three primary injections in infancy.

**MATERIALS AND METHODS**

**Subjects**

Infants scheduled to receive the first dose of pertussis vaccine were recruited in Campinas Public Health Centers, São Paulo, Brazil. A total of 247 infants were recruited to the study and randomized to receive three doses of wP\textsubscript{low} or wP vaccines at 2, 4 and 6 months of age. The preterm (gestational age <37 weeks) and the low birth weight newborns (weight <2500g), infants whose mothers were younger than 18 years old or had hepatitis B carrier status, infants with family history of tuberculosis, syphilis or Human Immunodeficiency Virus (HIV) infection were excluded. Exclusion criteria also included non compliance, receipt of immunoglobulin therapy or blood products, any immunodeficiency, malignancy, significant underlying disease or neurological impairment.
Vaccines

Vaccines were manufactured by Butantan Institute, São Paulo, Brazil. *Bordetella pertussis* cells were treated with an organic solvent and washed in order to perform LPS extraction. The culture was then detoxified by addition of 0.2 per cent formalin and bacterial biomass was obtained by tangential flow filtration to formulate wP<sub>low</sub> vaccine.

Each 0.5mL dose of both vaccines contained 4 protective units of pertussis, and 2 protective units of diphtheria and tetanus toxoid. The antigens were absorbed onto 1.25 mg of aluminum hydroxide, and 0.2 mg of thimerosal was used as a preservative. All infants received vaccine from the same batch. The vaccines were visually indistinguishable and identically packaged and were administered intramuscularly with the use of standard techniques.

Study design

This prospective, randomized, double-blind comparative trial was conducted between August 2006 and July 2007, following the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants’ parent or legal guardian before study procedures were initiated. The study protocol was approved by the Committee for Ethics in Research from UNICAMP, São Paulo, Brazil.
Antigens used in cell culture assays

Heat-killed *B. pertussis* suspension (Butantan Institute, lot IB-CIIn/P14/06), without thimerosal, was used at 5 x 10⁶ cells/mL. Phytohemagglutinin (PHA, Sigma, USA) was used as a positive control at 7.5 µg/mL.

T cell proliferation assay

Ten milliliters of heparinized peripheral blood were collected and used to evaluate immune responses to pertussis vaccination at 7 months of age. The protocol for proliferation assay was adapted from Gaines & Biberfeld. (20). Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation over Ficoll-Hypaque (Amersham Biosciences, USA), washed, diluted to 1 x 10⁶ cells/mL in RPMI 1640 medium (Sigma, USA) supplemented with 10% human AB serum (Sigma, USA), 1% glutamine (Sigma, USA) and 0.1% gentamycin and stimulated for 6 days with with heat-killed *B. pertussis*, phytohemagglutinin (PHA) or medium alone at 37°C with 5% CO₂ in round-bottomed 96-well tissue culture plates (NUNC, Denmark). After harvesting with 20mM ethylene diamine tetracetic acid (EDTA), samples were incubated with human immunoglobulin and then stained with anti-human CD3, CD4, CD8 and T cell receptor (TCR) pan γδ fluorescent antibodies (Beckman Coulter, USA) before acquisition (Epics XL-MCL flow cytometer, Beckman-Coulter, USA) and analysis (Expo, Software, Beckman- Coulter, USA). Isotype controls were used to discriminate positive populations (Beckman Coulter, USA). Only CD3⁺ T cells were
used in analysis. Forward and side scatters were used to gate on resting and blast lymphocytes. Dead cells were excluded from all analyses. CD4+, CD8+ and TCR γδ+ cells were identified in the gate of blast lymphocytes. Proliferation was measured by CD3+ percent blasts, in which basal proliferation was subtracted from *B. pertussis* and PHA-stimulated cultures.

**Cytokine quantification in supernatants**

For cytokine quantification assay, PBMC were diluted to 2 x 10⁶ cells/mL in supplemented RPMI and incubated for 48 hours in round-bottomed 96-well tissue culture plates with *B. pertussis*, PHA or medium alone at 37°C. Supernatants were collected and stored at -80°C. Interferon-γ (IFN-γ), Tumor Necrosis Factor-α (TNF-α), Interleukin 4 (IL-4) and Interleukin 10 (IL-10) levels were determined in duplicate by a two-Monoclonal Antibody sandwich Enzyme Linked Immunosorbent Assay (two-MAb ELISA) (R & D Systems) in flat-bottomed MultiSorp ELISA plates (NUNC, Denmark), according to the manufacturer’s protocols. Recombinant cytokine was used for the standard curve. The limit of detection was 15.6pg/mL for IFN-γ, and TNF-α, and 31.2pg/mL for IL-4 and IL-10.

**Quantitative determination of anti-PT IgG**

Quantitative determination of anti-pertussis toxin IgG (anti-PT ) was performed blindly on serum samples collected one month after the third dose of vaccine and stored at -80°C until tested. Using ELISA (29), anti-PT IgG levels were calculated in IU/mL by comparison with an U.S. reference human anti-serum (Food and Drug...
Administration, lot 3). To evaluate the titers, the values were transformed into decimal logarithm and then the mean and the limits of 95% IC were determined. Thereafter, the antilogarithm and the corresponding 95% IC were calculated.

**Quantitative determination of anti-Diphtheria and tetanus IgG**

*In vitro* tests for measuring tetanus and diphtheria antitoxin levels in serum from both vaccination groups were done by a standardized modified toxin-binding inhibition (TOBI) assay [41] at the Quality Control Service of Butantan Institute, São Paulo - Brazil.

**Adverse event monitoring**

Parents were asked to notify the study staff immediately by phone of any unexpected or severe reactions. A standardized questionnaire was used to collect information about any occurrence related to vaccination using scripted questions and definitions. After the first dose, parents’ compliance to adverse events monitoring was assessed by phone interview. At the second and third doses and blood sampling, compliance was assessed by the study nurses during Public Health Center visits.

**Statistical considerations**

Analyses were carried out with SPSS® for Windows (version 7.5.1, USA). Mann-Whitney and Friedman nonparametric tests were used in proliferation and cytokine analyses. Comparisons manifesting a two-tailed *p* value of <0.05 were
considered statistically significant. GraphPad Prism (version 4.0, GraphPad Software, USA) was employed for the figures.

To evaluate the anti-PT titres, calculation of geometric mean titres (GMTs) of antibodies were performed on $\log_{10}$ transformed data, reporting the antilogarithm. For each group, GMTs and 95% confidence intervals (CIs) were calculated. For comparison of the logarithm of the titres, Student’s t test was applied for independent samples. Comparisons manifesting a two-tailed $p$ value of $<0.05$ were considered statistically significant (25).

The differences in the proportions of seroprotection for diphtheria, tetanus and hepatitis B and 90% confidence intervals (IC 90%) were calculated, as recommended for non-inferiority studies (10, 36). Differences or ratios equal or lower than 10% were accepted as the limit for defining non-inferiority of low LPS cellular pertussis vaccine ($wP_{low}$). The null hypothesis of non-inferiority of $wP_{low}$ vaccine was accepted when the lower limit of the confidence interval was not lower than -10%.

Results

Study participants

Out of 247 infants initially selected, 234 participated in the entire study distributed as follows: 115 infants in the low LPS vaccine group (57 male and 58 female) and 119 infants in the whole-cell one (65 male and 54 female). Table 1 shows the mean and standard deviation values of the infants’ age at the three doses of vaccine and at blood sampling.
B. pertussis-specific T cell proliferation

T cell proliferation was measured by flow cytometry (Fig. 1). CD3+, CD4+ and CD8+ immunophenotyping was carried out in the cultures of 205 infants and TCR γδ+ blast cells were analyzed in 55 children (28 of the low LPS vaccine group).

Median background CD3+ proliferation was 2.3% in the wPlow group and 2.6% for wP. Net percent CD3+ blasts in cultures with B. pertussis were higher in the group vaccinated with wP (medians of 6.2% and 3.9% for wP and wPlow, respectively; p=0.029) but there was no difference in cultures stimulated with PHA (medians of 82.5% and 81.4%; p=0.166) (Fig. 2).

The frequencies of B. pertussis- and PHA-proliferating CD4+, CD8+ and γδ+ cells were similar between vaccination groups (Table 2). On the other hand, there was a significant difference between the T cell subpopulations for control, B. pertussis- and PHA-stimulated cultures (Friedman test, p<0.001), with higher percentages of γδ+ cells in the B. pertussis- ones.

B. pertussis-specific cytokine production

B. pertussis-specific cytokines in cell cultures of infants were determined by ELISA after 48 hours stimulation. The amount of cytokine secretion in supernatants was compared among the groups of vaccinees. Undetectable amounts of IL-4 were observed in stimulated and in unstimulated samples (negative controls). IFN-γ, TNF-α and IL-10 production (Fig. 3) were not different in B. pertussis- and PHA-stimulated cultures of infants vaccinated with wPlow or wP.
Anti-PT IgG titers

Pertussis antibody GMTs following three doses of \( wP_{\text{low}} \) or \( wP \) indicated robust responses for both vaccines (Table 3) and no statistically significant differences between the two groups were observed (Student’s t test, \( p = 0.464 \)).

Diphtheria and tetanus seroprotection

Table 4 shows the percentages and 95% confidence intervals of subjects protected to diphtheria and tetanus. As the lower limit of the confidence interval was not lower than -10% for the three studied parameters, the null hypothesis of non-inferiority of \( wP_{\text{low}} \) vaccine was accepted.

Adverse events

Within the first 2-3 days of each injection, the parents and legal guardians of the vaccinees reported local reactions, as erythema \( \geq 20 \text{mm} \) (\( wP_{\text{low}} \), 3.6% and \( wP \) 1.4%) and pain (\( wP_{\text{low}} \), 5.3% and \( wP \) 2.3%). Likewise, systemic reactions as body temperature \( \geq 39^\circ C \) were observed in both groups (\( wP_{\text{low}} \), 3.8% and \( wP \), 2.0%). Irritability was reported after 4.4% of \( wP_{\text{low}} \) and 2.9% of \( wP \) injections, respectively. Severe systemic adverse effects after each DTP vaccine dose were not reported. None of the participants withdrew from the study because of vaccine-related adverse events. There were no significant differences between \( wP_{\text{low}} \) and \( wP \) groups.
Discussion

Studies of immune responses to pertussis vaccines suggest that both B- and T-cell responses are elicited in humans (5, 7, 14) and mice (4, 33). Using a murine model of infection, it has been demonstrated that adoptive transfer of CD4+ T cells from immune mice confers protection from *B. pertussis* challenge in the absence of detectable Ab response but also passive Ab transfer protected mice from *B. pertussis* infection (27). In an attempt to better understand the CMI responses to pertussis vaccines in infants, we evaluated the T cell *in vitro* proliferation specific to *B. pertussis* at one month after the third dose of vaccine.

We used a flow cytometry-based lymphocyte proliferation assay that allowed the characterization of specific subpopulation expansion in response to *B. pertussis*. We found that infants vaccinated with both wP and wP_low exhibited similar frequencies of *B. pertussis*-proliferating CD4+, CD8+ and γδ+ cells, although total CD3+ proliferation in wP group was significantly higher.

We report here, for the first time that, in humans, high percentages of γδ+ cells are found in *B. pertussis* stimulated cultures (medians of 20.3% for wP and 16.8% for wP_low), suggesting that these cells may play an important role in pertussis-specific response. T cells expressing the γδ receptor were identified in the mid 1980s (11, 12,15, 39) and are disproportionately abundant within epithelial surfaces, including those in the lung (26). Their intraepithelial distribution and capacity to recognize nonprotein antigens, sometimes in a non-major histocompatibility complex-restricted fashion, has led to their consideration as part of the first line defense against pathogens (23). In humans, large expansion of γδ+ T cells after *Bacillus Calmette Guérin* vaccination and during infection with *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Brucella*...
The activation of γδ+ T lymphocytes can lead to IFN-γ production that is instrumental in the upregulation of both macrophage and natural killer (NK) cell functions central to early antibacterial protection prior to the αβ+ T cell response (23). IFN-γ also influences the downstream acquisition of a Th1 phenotype by αβ T cells (42). γδ+ T cells thus bridge the innate and acquired immune response by providing initial protection of epithelia from invasion and injury in instances where αβ T cells are not yet operational and then down-regulating the antigen-specific adaptive immune response after the danger has passed to minimize potential immune-mediated injury (40).

Although little is known about the importance of γδ+ T lymphocytes during B. pertussis infection, a role for these cells in the response of infected children has previously been suggested by the migration of circulating γδ+ T cells to the airways (9). Moreover, using a murine aerosol challenge model, Zachariadis and co-workers demonstrated that the absence of γδ+ T cells could influence the subsequent adaptive immune response to B. pertussis antigens, as evidenced by a shift from a Th1 to a Th2 type response against filamentous hemagglutinin (FHA) in γδ TCR -/- mice (48).

Th1 cytokines are associated with protection in various B. pertussis infection models, and in particular, in humans, protection from pertussis after infection or vaccination is determined by the presence of Th1 cytokines such as interleukin-12 (IL-12) and IFN-γ (37). Also, the clearance of B. pertussis or protection induced by wP vaccines is dependent on the production of Th1 cytokines, since IFN-γ or IFN-γ receptor-defective mice and mice depleted of NK cells, which infiltrate the lung and secrete IFN-γ early in infection, develop disseminating lethal infections (13).
In this study, ELISA measurements of cytokine levels in culture supernatants demonstrated significant increases in IFN-γ and TNF-α secretion by *B. pertussis* stimulated PBMC. Since there was no increase in IL-4 secretion, we speculate that the cytokine production in response to *B. pertussis* was more Th1-like for both vaccines. However, our assay did not permit the identification of the cells which are secreting these cytokines. Similar Th1 cytokine secretion profiles for adults (3) and children (6, 31, 37) have been reported for cellular pertussis vaccines. In contrast, T cells from children immunized with acellular pertussis vaccines can also secrete IL-5 following stimulation with *B. pertussis* antigens and generate a type 2 effector response (38).

Because of the bias against Th1-cell-polarizing cytokines, it was initially thought that the neonatal immune system was generally impaired or depressed. However, mounting evidence suggests that, under some circumstances, human neonates seem able to develop mature Th-cell responses, ranging from deficient or deviant to fully mature, depending on the conditions of antigen exposure (reviewed in ref. 1). Our results demonstrate that infants are able to mount Th1 responses to *B. pertussis* after wP or wP<sub>low</sub> administration.

The induction of protective Th1 responses by immunization with wP or by previous infection with *B. pertussis* has been associated with IL-12 production by macrophages or dendritic cells (DC), and this has been linked with LPS present in the wP preparations and in the live bacteria (30). LPS signaling through TLR4 in innate immune cells play a critical role in the generation of inflammatory cytokines, IL-12, IL-23, and IL-1, which direct the induction of Th-1 and Th-17 cells in mice immunized with wP. Furthermore, the cytokines secreted by these T cell subtypes promote bacterial killing by macrophages, a response that is further enhanced at the effector level by TLR4-mediated activation of macrophages. Thus, TLR4 plays a critical role in the
induction and in the effector phase of the protective cellular immune response to *B. pertussis* induced by vaccination (24), which could explain the lower proliferation of CD3+ T cells observed in wP_low group.

Additionally, the null hypothesis of non-inferiority of wP_low vaccine was accepted because wP_low and wP elicited a similar anti-PT IgG geometric mean titer and did not interfere in tetanus or diphtheria seroconversion. Theoretically, wP_low with 95% less LPS would have weaker adjuvant activity and therefore lower antibody response. In this sense we can understand the significantly higher total CD3+ proliferation in wP group as a result of the LPS linked to the host Toll-like receptor complex TLR4 / MD-2 and the subsequent activation of NF-κB (35). Because there is no defined parameter for pertussis seroprotection (18), we could not estimate differences in the proportions of protected infants.

Our surveillance system for severe adverse events following immunization was able to detect an excellent primer safety profile of wPlow and wP vaccines. DTP vaccine changes from manufacturer to manufacturer, because of the different *Bordetella pertussis* strains used for production. Some manufacturers even use two different strains. There is a rare, although serious risk of a severe adverse event related to the pertussis component of DTP. The risk of encephalopathy and convulsions varies according to the origin of the vaccine and the strain used for production. These risks have been estimated in 1/19,496 for febrile convulsions, 1/76,133 for convolution without fever and 3/10^6 for encephalopathy. (17, 19). Whole cell pertussis vaccine available in Brazil (licensed by Butantan Institute) has been administered free of charge to almost 100% of infants in the past 15 years. More than 50 million doses have been used, without major adverse effects. According to the Brazilian Ministry of Health pertussis incidence has declined from 10.8 in 1990 to 0.55 per 100,000 in 2005. In the...
same period, diphtheria incidence decreased from 0.45 to 0.01 per 100,000, and tetanus incidence from 1.0 to 0.25 per 100,000.

Since there was a similar pattern of response in both groups of vaccination, it is reasonable to assume that the low LPS cellular pertussis vaccine is capable to induce \textit{B. pertussis} specific response and, consequently, is immunogenic. Therefore, our data endorse further investigations with wP\textsubscript{low} vaccine. On the other hand, it must be recognized that, although low LPS cellular pertussis vaccine was similar to the conventional whole-cell one, based on Th1-polarized effector response and specific \(\gamma\delta^+\) T cell expansion the overall data did suggest that wP performed modestly better specific CD3+ cell proliferation.

\textbf{Acknowledgements}

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**Table 1.** Distribution of infants vaccinated with low LPS cellular pertussis (\(wP_{\text{low}}\)) or whole-cell pertussis (\(wP\)) vaccines by age (in months) at the three doses of vaccine and at blood sampling.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>n</th>
<th>Age (months) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{\text{st}}) dose</td>
<td>(wP_{\text{low}})</td>
<td>124</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(wP)</td>
<td>123</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>2(^{\text{nd}}) dose</td>
<td>(wP_{\text{low}})</td>
<td>121</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(wP)</td>
<td>121</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>3(^{\text{rd}}) dose</td>
<td>(wP_{\text{low}})</td>
<td>120</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>(wP)</td>
<td>119</td>
<td>6.3 ± 0.4</td>
</tr>
<tr>
<td>Blood sampling</td>
<td>(wP_{\text{low}})</td>
<td>117</td>
<td>7.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(wP)</td>
<td>119</td>
<td>7.7 ± 0.6</td>
</tr>
</tbody>
</table>

\((a): number of individuals; (b): standard deviation.\)
Table 2: Percentages of CD4+, CD8+ and γδ+ blast cells determined by flow cytometry in PBMC cultures incubated with medium alone (control), *B. pertussis* or phytohemagglutinin (PHA) from infants vaccinated with low LPS cellular pertussis (wP<sub>low</sub>) or whole-cell pertussis (wP) vaccines.

<table>
<thead>
<tr>
<th>Median % of T cell (95% CI)&lt;sup&gt;®&lt;/sup&gt;</th>
<th>wP&lt;sub&gt;low&lt;/sub&gt;</th>
<th>wP</th>
</tr>
</thead>
<tbody>
<tr>
<td>n&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Control</td>
<td>B. pertussis</td>
</tr>
<tr>
<td>CD4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>102</td>
<td>53.2 (49.6 – 58.3)</td>
</tr>
<tr>
<td>CD8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>102</td>
<td>29.6 (25.6 – 33.1)</td>
</tr>
<tr>
<td>γδ&lt;sup&gt;+&lt;/sup&gt;</td>
<td>28</td>
<td>5.7 (4.0 – 7.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Peripheral blood mononuclear cell; <sup>b</sup>: 95% confidence interval for median; <sup>c</sup>: number of individuals; <sup>d</sup>: phytohemagglutinin
Table 3. Geometric mean titer (GMT) and 95% confidence interval (CI) for anti-PT IgG serum levels of infants vaccinated with three doses of low LPS cellular pertussis (wPlow) or whole cell pertussis (wP).

<table>
<thead>
<tr>
<th>Group</th>
<th>n(^a)</th>
<th>GMT (95% CI)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wPlow</td>
<td>114</td>
<td>12.65 IU/mL [10.46 – 15.30]</td>
</tr>
<tr>
<td>wP</td>
<td>116</td>
<td>14.07 IU/mL [11.36 – 17.42]</td>
</tr>
</tbody>
</table>

\(^{a}\): number of individuals; \(^{b}\): geometric mean titer and 95% confidence interval
Table 4. Geometric mean titer (GMT) and 95% confidence intervals (CI) for IgG Anti-Tetanus and Anti-Difteria serum levels of seven-month-old infants vaccinated with three doses of low LPS ($wP_{low}$) or whole-cell pertussis ($wP$) vaccines.

<table>
<thead>
<tr>
<th></th>
<th>$wP_{low}$</th>
<th>$wP$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Tetanus $\geq 0.10$ IU/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td>107/112</td>
<td>111/113</td>
</tr>
<tr>
<td>% [95% CI]</td>
<td>95.5 [89.9 – 98.5]</td>
<td>98.2 [93.8 – 99.8]</td>
</tr>
<tr>
<td>Anti-Diphtheria $\geq 0.10$ IU/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n/N</td>
<td>104/113</td>
<td>107/112</td>
</tr>
<tr>
<td>% [95% CI]</td>
<td>92.0 [85.4 – 96.3]</td>
<td>95.5 [89.9 – 98.5]</td>
</tr>
</tbody>
</table>

n: number of subjects considered protected; N: total of subjects in evaluation
Vaccine

Figure 1:
Figure 2:

![Graph showing CD3+ percent blasts with p = 0.029, n = 102 for WP_low and n = 103 for WP.](image)
Figure 3A:
Figure 3B:

![Graph showing TNF-α concentration (pg/mL)]

- Control
- Bp
- PHA

**wP**

- **wP**
- **wP**

**n**

- 32
- 34
Figure 3C:


IL-10 concentration (pg/mL)
Fig. 1: Flow cytometry of unstimulated (control), *B. pertussis* (Bp) and phytohemagglutinin (PHA) stimulated peripheral blood mononuclear cells of a 7-month-old infant vaccinated with low lipopolysaccharide cellular pertussis. After gating on CD3⁺ cells (A), events were analyzed for size and complexity (FS and SS gates), from which resting and blast lymphocytes were separated (B). T lymphocyte subsets (CD4⁺, CD8⁺ e TCR δγ⁺) were then verified in blast lymphocytes.

Fig. 2: Distribution of CD3⁺ percent blasts determined by flow cytometry of *B. pertussis* (Bp: ■) and phytohemagglutinin (PHA: ▲) stimulated peripheral blood mononuclear cells from low lipopolysaccharide cellular pertussis (wPₗow) or whole-cell pertussis (wP) vaccinated infants. Medians (indicated by bars) of CD3⁺ blasts in Bp-stimulated cultures were 3.9% and 6.2% for wPₗow and wP vaccines, respectively. Medians of CD3⁺ blasts in PHA-stimulated cultures were 81.4% and 82.5%, respectively; n: number of individuals.

Fig. 3: IFN-γ (A), TNF-α (B) and IL-10 (C) concentration determined by ELISA (values are given in picograms per milliliter) by peripheral blood mononuclear cells in cultures without stimuli (control: ■), with *B. pertussis* (Bp: ▲) or phytohemagglutinin (PHA: ●) stimulation in infants immunized with low lipopolysaccharide cellular pertussis (wPₗow) or whole-cell pertussis (wP) vaccines. Bars indicate medians and Mann-Whitney test was used to verify significances; n: number of individuals.