Development of Recombinant Pertussis Vaccines

Pham Hong Thai, CEO
BioNet-Asia Co., Ltd, Bangkok, Thailand

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Pertussis (whooping cough) is an important cause of death in infants worldwide, and continues to be a public health concern despite high vaccination coverage. In 2013, according to WHO estimates, pertussis was still causing around 63 000 deaths in children aged <5 years. Two types of pertussis vaccines are available: wP vaccines and aP vaccines.

A switch from wP to aP vaccines for the primary schedule should only be considered if additional periodic booster or maternal immunization can be assured and sustained. National programmes currently administering wP vaccination should continue to use wP vaccines for primary vaccination series. National programmes currently using aP vaccine may continue using this vaccine but should consider the need for additional booster doses and additional strategies such as maternal immunization in case of resurgence of pertussis.
Resurgence of Pertussis
An Increasing Concern Worldwide

- Waning immunity
- Genetic shifts of circulating Bp strains

Table 1. Possible Vaccination Strategies to Control the Resurgence of Pertussis

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Return to the use of wcP</td>
<td>Probably unacceptable</td>
</tr>
<tr>
<td>Develop less-reactogenic wcP</td>
<td>Not yet done</td>
</tr>
<tr>
<td>Maternal vaccination to provide transplacental antibody to protect newborn</td>
<td>Now generally recommended</td>
</tr>
<tr>
<td>Vaccination of newborn contacts</td>
<td>Difficult to obtain complete coverage</td>
</tr>
<tr>
<td>(cocoon strategy)</td>
<td></td>
</tr>
<tr>
<td>More frequent boosters with acP</td>
<td>Costly and difficult to put in place</td>
</tr>
<tr>
<td>Change antigens in acP to those from currently circulating strains</td>
<td>Uncertain effect</td>
</tr>
<tr>
<td>Increase quantities of current antigens</td>
<td>Would require large trials</td>
</tr>
<tr>
<td>Inactivate PT by genetic mutation or milder chemical</td>
<td>Probably advisable to increase immunogenicity</td>
</tr>
<tr>
<td>Add new virulence factors</td>
<td>Would require large trials</td>
</tr>
<tr>
<td>Use stronger adjuvants</td>
<td>May require large trials</td>
</tr>
<tr>
<td>Administer live attenuated <em>Bordetella pertussis</em> intranasally</td>
<td>Early development Probably best as a boost strategy</td>
</tr>
</tbody>
</table>

Abbreviations: acP, acellular pertussis vaccine; PT, pertussis toxin; wcP, whole-cell pertussis vaccine.

Source: Plotkin A. (2013) Clinical Infectious Diseases
Bordetella pertussis
Pathogenesis

• PT
  • Principal toxin secreted by Bp, 5 subunits, A-B structure
  • Many pathologic effects mediated by ADP ribosylation of G protein effectors

• FHA
  • Filamentous adhesion factor

• PRN (“6gK”)
  • Impurity present in Japanese T-type vaccines
  • RGD sequences promoting adhesion to cells

• Agg 2+3
  • or Fimbriae
Pertussis Vaccines
Three Types of Vaccines

- **Whole-cell Pertussis vaccines (wP)**

- **Acellular Pertussis vaccines using chemically detoxified Pertussis Toxin (cPT)**
  - Co-purified antigens (Asia)
  - Individually purified antigens (Western countries)

- **Recombinant Pertussis vaccines**
  - Live-attenuated (nasal route)
  - Inactivated
    - Genetically-detoxified Pertussis Toxin
    - Recombinant antigens such as PT, ACT, PRN...
Call for New Pertussis Vaccines
Genetically-Inactivated PT, the Solution?

The Diphtheria and Pertussis Components of Diphtheria-Tetanus Toxoids-Pertussis Vaccine Should Be Genetically Inactivated Mutant Toxins

John B. Robbins, Rachel Schneerson, Birger Trolle, Kienho Sato, Yoji Sato, Rino Rappuoli, and Jerry M. Keith
National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland; Department of Pediatrics, University of Saitama, Saitama, Japan.

Relative Contribution of Th1 and Th17 Cells in Adaptive Immunity to Bordetella pertussis: Towards the Rational Design of an Improved Acellular Pertussis Vaccine

Pádraig J. Ross, Caroline E. Sutton, Sarah Higgins, Aileen C. Allen, Kevin Walsh, Alicja Misliak, Ed C. Lavelle, Rachel M. McCoughlin, Kingston H. G. Mills
1 Immune Regulation Research Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland; 2 Adjuvant Research Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland; 3 Host Pathogen Interactions Group, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland.

The rise in pertussis cases urges replacement of chemically-inactivated with genetically-inactivated toxoid for DTP

John B. Robbins, Rachel Schneerson, Jerry M. Keith, Joseph Shiloach, Mark Miller, Birger Trolle.

Genetically Detoxified Pertussis Toxin Induces Th1/Th17 Immune Response through MAPKs and IL-10-Dependent Mechanisms

Maria Nasso, Giorgio Fedele, Fabiana Spensieri, Raffaella Palazzo, Paolo Costantino, Rino Rappuoli, and Clara Maria Ausiello.
Genetically Detoxified Pertussis Toxin
A Non-Toxic and Superior Immunogen

**rPT** is a PT devoid of toxicity while maintaining the other properties of the native PT.

**cPT** introduces dramatic changes on the toxin surface.

- Chemical treatment can destroy up to 80% of surface epitopes
- The rPT preserves the epitopes for T-cell binding significantly better than cPT.

Source: Ibsen H, 1996
Chiron Pediatric DTaP containing 5 µg rPT
84% Efficacy in US NIAID-Sponsored Italian Efficacy Trial

Table 1. Efficacy trials of acellular pertussis vaccine

<table>
<thead>
<tr>
<th>Study site (Period)</th>
<th>Vaccine</th>
<th>Manufacturer</th>
<th>Component (µg P/dose)</th>
<th>Dose</th>
<th>VE(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweden (1992–1995)</td>
<td>DTaP(2)</td>
<td>SKB</td>
<td>25 25 0 0</td>
<td>3</td>
<td>59(51–66)</td>
</tr>
<tr>
<td></td>
<td>DTaP(5)</td>
<td>CLL</td>
<td>10 10 3 5</td>
<td>3</td>
<td>85(81–89)</td>
</tr>
<tr>
<td></td>
<td>DTwP</td>
<td>CLI</td>
<td>— — — —</td>
<td>3</td>
<td>48(37–58)</td>
</tr>
<tr>
<td>Sweden (1991–1994)</td>
<td>DTaP(1)</td>
<td>NAV</td>
<td>40 0 0 0</td>
<td>3</td>
<td>71(63–78)</td>
</tr>
<tr>
<td>Sweden (1986–1990)</td>
<td>aP(2)</td>
<td>Biken/JNIH6</td>
<td>23 23 0 0</td>
<td>2</td>
<td>92(83–96)</td>
</tr>
<tr>
<td>Italy (1992–1993)</td>
<td>DTaP(3)</td>
<td>CB</td>
<td>5 2.5 2.5 0</td>
<td>3</td>
<td>84(76–90)</td>
</tr>
<tr>
<td></td>
<td>DTaP(3)</td>
<td>SKB</td>
<td>25 25 8 0</td>
<td>3</td>
<td>84(76–90)</td>
</tr>
<tr>
<td></td>
<td>DTwP</td>
<td>CLI</td>
<td>— — — —</td>
<td>3</td>
<td>86(44–92)</td>
</tr>
<tr>
<td>Germany (1993–1995)</td>
<td>DTaP(2)</td>
<td>B/CL</td>
<td>23 23 0 0</td>
<td>3</td>
<td>82(68–90)</td>
</tr>
<tr>
<td></td>
<td>DTwP</td>
<td>BW</td>
<td>— — — —</td>
<td>3</td>
<td>96(87–99)</td>
</tr>
<tr>
<td>Germany (1992–1994)</td>
<td>DTaP(3)</td>
<td>SKB</td>
<td>25 25 0 0</td>
<td>4</td>
<td>89(77–95)</td>
</tr>
<tr>
<td>Germany (1991–1994)</td>
<td>DTaP(4)</td>
<td>L/T</td>
<td>3.2 34.4 1.6 0.8</td>
<td>4</td>
<td>82(75–89)</td>
</tr>
<tr>
<td></td>
<td>DTwP</td>
<td>L</td>
<td>— — — —</td>
<td>4</td>
<td>91(86–96)</td>
</tr>
<tr>
<td>Senegal (1990–1994)</td>
<td>DTaP(2)</td>
<td>PM</td>
<td>25 25 0 0</td>
<td>3</td>
<td>74(52–87)</td>
</tr>
<tr>
<td></td>
<td>DTwP</td>
<td>PM</td>
<td>— — — —</td>
<td>3</td>
<td>91(79–96)</td>
</tr>
</tbody>
</table>


Source: Y. Sato and H. Sato, Biologicals, 1999
GSK (Novartis) Adult aP/TdaP in Phase I Study

Immunogenicity Results at Day 30 post vaccination

GMT anti-PT Antibody

GMT anti-FHA Antibody

https://clinicaltrials.gov/ct2/show/NCT01529645
BioNet Recombinant Acellular Pertussis Vaccines
BioNet Pertussis Project
Timelines and Status

- R&D project with Mahidol University for Recombinant Pertussis strain
- Development of Recombinant Pertussis Vaccine
- Project completion with rPT
- Patent filing
- Phase I/II
- Phase II/III
- Preclinical study
- Manufacturing of clinical materials
- GMP Master cell bank
- Proof-of-concept study

Design of *Bordetella pertussis* Construct

Scar-free Recombinant Pertussis Strain (PCT Publication: WO 2013/141823)

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**BioNet modified Bp strain**

- no antibiotic resistance marker remaining after strain construction

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- Mutations: ARG9 to LYS9 and GLU129 to GLY129
- Resulting in the loss of its catalytic and toxic effects

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Source: Buasri et al. (2012) *BMC Microbiology* 12:61
BioNet Recombinant Pertussis Toxin
Loss of Toxicity by Genetic Modification

PT-Specific Toxicity Test in CHO cells

- Toxicity of wild type PT by clustering of CHO cells in dose-dependent manner.
- Reduced toxicity of recombinant PT by a factor of $5 \times 10^5$ to $1 \times 10^6$
- Purified rPT was successfully inactivated by mutation at 9K/129G at S1 subunit resulting in loss of catalytic toxicity of PT.

Source: Buaasri et al. (2012) BMC Microbiology 12:61
Clinical Study of BioNet rP/TdrP

Phase I/II Study Procedures Overview

- **Objective**: To assess safety and immunogenicity of a single injection of BNA’s rP or BNA’s TdrP or Adacel® (Sanofi Pasteur) vaccines

- **Study Population**: Healthy adult volunteers (Male & Female), 18-35 years of age

- **Principle Investigator**: Prof. Chukiat Sirivichayakul, Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
BioNet Clinical Study

Summary

- Overall, subjects in BNA’s rP & TdrP vaccine groups had similar local, systemic post-immunization reactions and AEs than subjects in the Adacel® group (data not shown).

- One month after vaccination
  
  • ELISA anti-PRN GMTs were similar in BNA’s rP & TdrP groups and in Adacel® group.

  • ELISA anti-PT and anti-FHA GMTs were statistically significantly higher in BNA’s rP & TdrP groups than in Adacel® group.
BioNet rPT in patch soon in Phase I Study
Preclinical Results Published on June 9th, 2015 in Vaccine

Needle-free and adjuvant-free epicutaneous boosting of pertussis immunity: Preclinical proof of concept

Beatris Mastelic Gavillet a,∗, Lucie Mondoulet b, Véronique Dheiff b, Christiane Sigrid Eberhardt a, Floriane Auderset a, Hong Thai Pham c, Jean Petre c, Paul-Henri Lambert a, Pierre-Henri Benhamou b, Claire-Anne Siegrist a

a World Health Organization Collaborating Center for Vaccine Immunology, Departments of Pathology-Immunology and Pediatrics, University of Geneva, 1211 Geneva, Switzerland
b DBV Technologies, Green Square, 80/84 rue des Meuniers, 92220 Rungis, France
c BioNet-Asia Co., Ltd., 15 Udomsuk 37, Sukhumvit 103, Bangjak, Phakanong, Bangkok 10260, Thailand