Vaccines based on genetically detoxified PT-9K/129G

Mariagrazia Pizza

Pertussis: biology, epidemiology and prevention

Annecy, Nov 13th, 2015
3D organization of PT structure
Catalytic residues in wild type (Arg 9 and Glu 129) and mutated S1 (Lys 9 and Gly 129)
Predicted effects of detoxification on PT structure

- No reversion to toxicity
- B and T cell epitopes conserved
- Higher immunogenicity

Arg and Lys residues, blue and red respectively
Recognition of genetically or chemically detox. PT by neutralizing MAbs

Titers are expressed as anti-detoxified PT ELISA to anti-native PT ELISA ratio. PTd = PT detoxified by 0.35% formaldehyde treatment; PTg = PT genetically detoxified (PT-9K/129G). Ibsen, P. H. (1996). Vaccine.
PT neutralization assay confirms the superiority of genetically detoxified PT

PT neutralization assay
- Active PT induces a *clustered phenotype* in CHO cells
- aP antibodies are able to inhibit the clustering
- Titers: reciprocal of the highest dilution able to inhibit cell clustering

Genetically detoxified PT elicits antibodies with higher neutralizing activity (mouse)
PT9K/129G induces higher protection in the animal models

aP-mediated protection depends on degree of formylation of the vaccine antigens

protection from colonization correlates with vaccine efficacy; protection mediated by Th1/Th17-dependent mechanisms
PT immunogenicity in humans: NIH trial

PT ELISA units/μg protein

Genetically
Chemically
Detoxified PT
PT immunogenicity NIH trial: dose response

Increasing PTg dose will lead to a superior vaccine
# 1990s Italian Efficacy Study: Design

N° of subjects enrolled = 14751; Multicenter trial: 62 centers - in 4 regions; Schedule: 2-4-6-months.

<table>
<thead>
<tr>
<th>Component</th>
<th>SmithKline DTaP</th>
<th>Biocine DTaP</th>
<th>Connaught (US) DTwP</th>
<th>Biocine DT (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PT</strong>: Inactive Pertussis Toxin (µg)</td>
<td>25</td>
<td><strong>5</strong> (genetically)</td>
<td>Pertussis*</td>
<td>--</td>
</tr>
<tr>
<td><strong>FHA</strong>: Filamentous hemagglutinin (µg)</td>
<td>25</td>
<td><strong>2.5</strong></td>
<td>Pertussis*</td>
<td>--</td>
</tr>
<tr>
<td><strong>PRN</strong>: Pertactin (µg)</td>
<td>8</td>
<td><strong>2.5</strong></td>
<td>Pertussis*</td>
<td>--</td>
</tr>
<tr>
<td><strong>D</strong>: Diphtheria Toxoid (flocculation units)</td>
<td>25</td>
<td><strong>25</strong></td>
<td>6.65</td>
<td>25</td>
</tr>
<tr>
<td><strong>T</strong>: Tetanus Toxoid (flocculation units)</td>
<td>10</td>
<td><strong>10</strong></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Aluminum-Salt Adjuvant</td>
<td>Aluminum Hydroxide</td>
<td>Aluminum Hydroxide</td>
<td>Aluminum Phosphate</td>
<td>Aluminum Hydroxide</td>
</tr>
<tr>
<td>Weight of ionic Al (mg)</td>
<td>0.5</td>
<td>0.35</td>
<td>0.15</td>
<td>0.7</td>
</tr>
<tr>
<td>Preservative</td>
<td>2-Phenoxyethanol</td>
<td>Thimerosal</td>
<td>Thimerosal</td>
<td>Thimerosal</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>2.5</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*5.7 IU per dose by mouse intracerebral challenge test, as determined by manufacturer

Greco D et al. NEJM 1996)
### 1990s Italian Efficacy Study: Immunogenicity

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>SmithKline DTap</th>
<th>Biocine DTaP</th>
<th>Connaught (US) DTwP</th>
<th>Biocine DT (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GMT (95% CI)</td>
<td>RESPONSE (%)</td>
<td>GMT (95% CI)</td>
<td>RESPONSE (%)</td>
</tr>
<tr>
<td>EIA for IgG to PT (units/ml)</td>
<td>51.3</td>
<td>94.5</td>
<td>94.4</td>
<td>96.7</td>
</tr>
<tr>
<td>EIA for IgG to FHA (units/ml)</td>
<td>147.0</td>
<td>85.1</td>
<td>52.6</td>
<td>60.5</td>
</tr>
<tr>
<td>EIA for IgG to PRN (units/ml)</td>
<td>274.2</td>
<td>96.6</td>
<td>136.6</td>
<td>95.9</td>
</tr>
<tr>
<td>PT neutralization by CHO</td>
<td>230.0</td>
<td>67.8</td>
<td>787.6</td>
<td>93.6</td>
</tr>
<tr>
<td>D</td>
<td>96.6</td>
<td>98.8</td>
<td>92.9</td>
<td>98.8</td>
</tr>
<tr>
<td>T</td>
<td>99.8</td>
<td>100</td>
<td>99.1</td>
<td>100</td>
</tr>
</tbody>
</table>

**Greco DNEJM 1996.**

EIA= enzyme linked immunoassay, CI= confidence interval, GMT= geometric mean titer, CHO= chinese hamster ovary assay
### 1990s Italian Efficacy Study: Efficacy

<table>
<thead>
<tr>
<th>VACCINE</th>
<th>EFFICACY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biocine DTaP</td>
<td>84.2 (76.2 – 89.7)</td>
</tr>
<tr>
<td>SmithKline DTap</td>
<td>83.9 (75.8 – 89.4)</td>
</tr>
<tr>
<td>Connaught (US) DTwP</td>
<td>36.1 (14.2-52.1)</td>
</tr>
</tbody>
</table>
PT9K/129G (at a dose of 1/5 of the chemically detox PT) was able to induce
• earlier protection from disease, before completion of the full vaccination schedule
• longer protection, in the later phase, when the titers of circulating antibody are declining
The need of Pertussis Booster Vaccines

- Significant resurgence of notified pertussis in the past decade despite global vaccination with high uptake
- Highest burden in infants under 6 months and persons over 10 years

(Source: http://www.cdc.gov/pertussis/downloads/pertussis-surveillance-report.pdf )

Reported pertussis incidence by age group: 1990-2012*

- Neonates: most vulnerable
- Infants: increasing incidence

* Maternal Booster

neonates

* Infant Booster
A Phase I, Randomized, Controlled, Observer-Blind, Dose-Ranging Study of Acellular Pertussis and Tetanus-Diphtheria-Acellular Pertussis Booster Vaccines in Adults Aged 18 to 40 Years
## V113_01: Study design

**Visit** | **Day 1** | **Day 30**
---|---|---
### Vaccine Group
<table>
<thead>
<tr>
<th>No of Subjects</th>
<th>Antigen Doses</th>
<th>Td-pur®</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>FHA</td>
<td>PRN</td>
</tr>
</tbody>
</table>
1 (NVD aP booster) | 42 | 1 | 1 | 2 | 0 | 0 | Td-pur® |
2 (NVD aP booster) | 42 | 2 | 2 | 4 | 0 | 0 | Td-pur® |
3 (NVD aP booster) | 42 | 4 | 4 | 8 | 0 | 0 | Td-pur® |
4 (NVD Tdap booster) | 42 | 1 | 1 | 2 | 2 | 5 | Saline |
5 (NVD Tdap booster) | 42 | 2 | 2 | 4 | 2 | 5 | Saline |
6 (NVD Tdap booster) | 42 | 4 | 4 | 8 | 2 | 5 | Saline |
7 (NVD Tdap booster) | 42 | 1 | 1 | 2 | 4 | 5 | Saline |
8 (NVD Tdap booster) | 42 | 2 | 2 | 4 | 4 | 5 | Saline |
9 (NVD Tdap booster) | 42 | 4 | 4 | 8 | 4 | 5 | Saline |
10 (Licensed comparator Tdap booster) | 42 | 8 | 8 | 2.5 | 2.5 | 5 | Saline |

### Blood draw
- Day 1
- Day 8
- Day 30
- Day 180
- Day 365
**Non-inferiority**

**Higher titers with less Ag**
**Key Learnings day 365**

- PT: Novartis aP/Tdap groups showed more sustained antibody persistency as compared to a licensed booster vaccine. All 4 mcg PT 9K/129G dosages were statistically superior compared to PT chemically detoxified.
Day 365 – aP kinetics over time

PT GMR kinetic aP vs. Licensed Vaccine

GMR

Days

aP 1-1-2  aP 2-2-4  aP 4-4-8  Boostrix
V113 Phase I study: Conclusions

– All investigational vaccines were well tolerated with no safety concerns identified.

– 30 days post-vaccination PT9K/129G formulations induced anti PT antibodies at higher level compared to the PT chem. detox., despite lower antigen doses.

– Antibody persistence (180/365 days) was evident in all groups, but waning of anti-PT antibodies was slower in PT9K/129G, as compared to the PT chem. detox. vaccinated subjects.
Conclusions

– Only genetic inactivation of pertussis toxin leads to a vaccine antigen that maintains all neutralizing epitopes
– Vaccines containing PT9K/129G outperformed vaccines containing chemically inactivated PT in pre-clinical infection models and in clinical trials even at lower antigen concentrations
– Future pertussis vaccine formulations should include the genetically inactivated PT-9K/129G instead of its chemically activated counterparts
The Pertussis Team

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