

WHOLE CELL PERTUSSIS VACCINE Standardization & Control

Dr. Elwyn Griffiths
EDQM-ECVAM Meeting
Geneva 16 March 2005



History

- 1906 - Isolation and identification of *Bordetella pertussis* as causative agent of pertussis by Bordet & Genou
- 1914 “whooping cough vaccine” listed in New and Non Official Remedies, USA
- 1920s – 1930s – various experimental vaccines tested in children
- 1940s -1950s – more clinical trials in USA and UK, including the pivotal MRC series



History

- Early trials – sometimes indication of efficacy, sometimes not: also of toxicity
- Problem – no accepted methods for assessing **toxicity** nor **potency** of preparation **PRIOR** administration
- Majority of trials plain vaccine, large bacterial content, large volumes (total 7 ml , 3 - 5 injections !!)



Standardization and Control

- 1930s, 1940s, 1950s: experience gained of growing *B pertussis*, killing, detoxifying and preserving cell suspensions
- Realization NEED to standardize and control manufacture of vaccine
- **Bacterial content (type and quantity) :**
POTENCY: Toxicity
- Possibility of producing effective vaccine became an achievable goal





Standardization and Control

- Use of Phase I *B pertussis* ONLY
(Leslie & Gardner 1931: 1984 Weiss & Falkow explained molecular mechanism - loss of virulence factors)
- Opacity Standard - bacterial content
- Excessive toxicity - mouse weight gain test became widely used
- POTENCY



Potency Test

- **TWO** candidate tests considered
- Intracerebral mouse protection test
(Kendrick et al 1947) (USA)
- Agglutinin production test in mice
(Evans and Perkins 1953) (UK)





Correlates of Protection

- MRC Trials (1951, 1956) with large number of vaccines and children showed :
- Substantial differences in protection in children
- Substantial degree correlation between activity of vaccines in protecting children and tests in mice
- **Protecting mice against intracerebral challenge (Kendrick) AND production of agglutinins in mice (and children) correlated with clinical protection**



Correlates of Protection

- Further MRC trial (1959) (13000 children) undertaken to include a novel pertussis vaccine - Pillemer et al (1954)
- Pillemer vaccine - sonic disintegration of *B pertussis*, adsorption of extract on to autoclaved human red cell stromata – first acellular vaccine





Correlates of Protection

- **Whole cell vaccines** good correlation -
 - * protection in children
 - * potency in mouse protection test
 - * agglutinin production test in mice



Correlates of Protection

- **Pillemer vaccine**
 - * protected well in children,
 - * protected well in Kendrick test
 - * did **NOT** show good correlation with agglutinin responses in either mice or children



Correlates of Protection

- “Agglutinin production cannot always be taken as evidence of protective activity” (MRC 1959)
- “Considered mouse protection test was the most satisfactory in assessing prophylactic activity”
- Rest is history



Aftermath

- Intracerebral Mouse Protection Test became official potency assay (WHO Requirements 1964)
- Pillemer vaccine abandoned – too toxic
- Later work suggests poor performance of Pillemer vaccine in mouse agglutinin test due to use of test strain of different serotype. Claimed when homologous strain used the sera were in fact agglutinin positive (Preston, Pittman)



MRC Trials Recognised

- although certain laboratory tests might give results paralleling protection in children
- does NOT necessarily mean that these tests measure DIRECTLY factor(s) responsible for protecting children
- Only correlates of protection




Intracerebral Mouse Protection

Test (Kendrick Test)

- Groups of mice immunized with serial dilutions of test and reference vaccine
- Intraperitoneal injection of vaccine
- Intracerebral challenge 14 -17 days later with 20-24hr culture of *B pertussis* strain 18323
- Mice observed for lethal effects over 14 days
- Potency estimated in terms of International Units by parallel line assay





Intracerebral Mouse Protection Test (Kendrick Test)

- Essentially same as Kendrick et al 1947
- Differences are in detail to ensure better data for statistical analysis.
- Weight of mice (>10 g, <18 g and within one test should not differ > 4 g from each other): 16 mice per group: humane end points
- Challenge strain 18323 from master and working seed, 20-24 h culture



Intracerebral Mouse Protection Test (Kendrick Test)

- Potency estimated in terms of International Units
- WHO Requirements – vaccine passes if results of statistically valid test shows estimated potency is NOT LESS than 4.0 IU per Single human dose, with lower fiducial limit (P = 0.95) of the estimated potency not less than 2.0 IU



Potency requirements

- By 1957 British Reference Vaccine was established: pertussis immunization widespread in UK
- Incidence of disease declined to 1962
- Epidemic of 1963-1964 cast some doubt that vaccines were as effective as those in the MRC clinical trials.
- PHLS survey strongly suggested some vaccines then in use were ineffective.



Potency requirements

- Although vaccines checked against British Reference Vaccine it was not until 1964 that WHO required 4 IU per single human dose
- Check found British Standard only 2.1 IU - well below new international requirement
- Discrepancy quickly corrected (Perkins 1969)– since 1966 only vaccines with potency of 4 IU or more used in UK



The Critics

- Animal welfare
- Technically difficult / Reproducibility
- **Unnatural challenge route**
- “Peculiar” challenge strain - most fresh isolates of *B pertussis* unable to establish intracerebral infection in mice . Strain 18323 does
- Potency test took no account of serotype specificity of vaccine or challenge strain



Challenge route

- **Support** for intracerebral route (Standfast 1958)
- Involves localization of bacteria on ciliated cells of the ependymal lining of ventricles
- Model involves ciliated cells like respiratory tract – but wrong location



The Critics

- Animal welfare
- Technically difficult / Reproducibility
- Unnatural challenge route
- “Peculiar” challenge strain - fresh isolates of *B pertussis* unable to establish intracerebral infection in mice . 18323 does
- **Potency test took no account of serotype specificity of vaccine or challenge strain**



Potency and Agglutinogens

- **Second factor** might have contributed to poor performance of British vaccines 1962 - 1967
- Relative abundance of different *B pertussis* serotypes had changed
- Pre 1958 serotypes 1,2 and 1,2,3 predominated
- By 1963 -1964 serotypes 1,3 predominated.
- One vaccine in common did not contain 1, 3 organisms
- Suggested a type 1,2 vaccine could not protect against infection with type 1,3 organisms





Potency and Agglutinogens

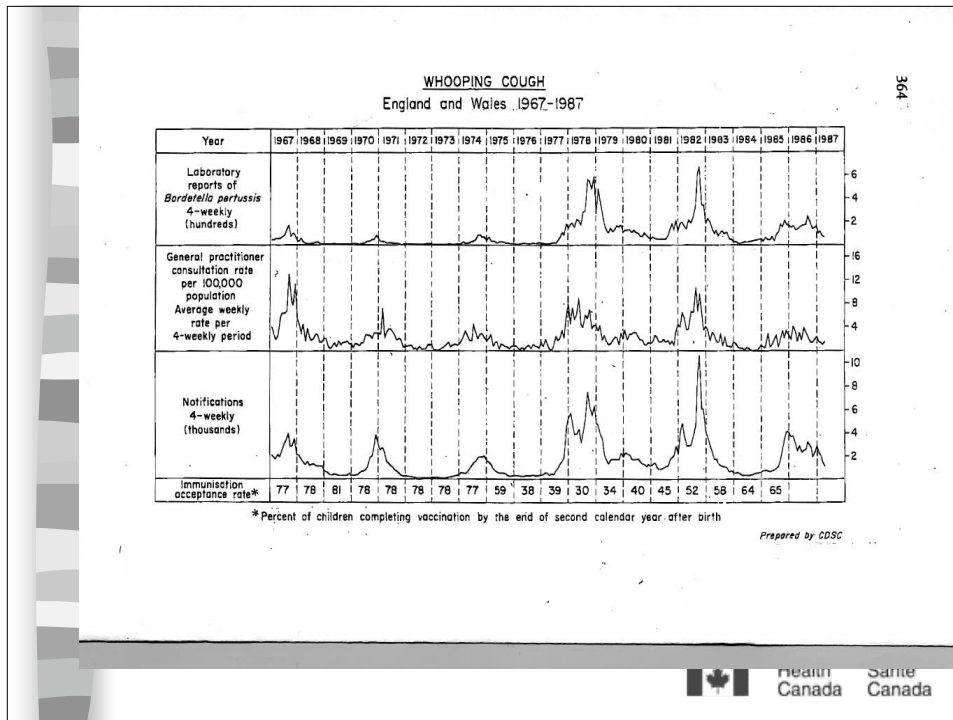
- New requirement in UK after 1966 – inclusion of *B pertussis* serotype 3.
- WHO Requirements of 1979 recommended strains used for vaccine production contain serotypes 1,2,3
- WHO Requirements of 1990 introduce test to confirm agglutinogens 1, 2, and 3 present in vaccine before adjuvant added
- Yet see protection with aP vaccines with no agglutinogens



Maintaining Protective Efficacy

- Concern in UK about alleged vaccine induced serious neurological events
- Led to massive decline in vaccine acceptance rate in UK (late 1970s /1980s) – from 80% to 30%
- Re-emergence of disease
- Stopping use of wP in Sweden and Japan led to similar resurgence of disease
- Gradual reintroduction of wP in UK > again reduction of disease
- Latest clinical evaluation of wP vaccine AT LEAST 85% efficacy





The Critics

- Animal welfare
- **Technically difficult / Reproducibility**
- Unnatural challenge route
- “Peculiar” challenge strain - fresh isolates of *B pertussis* unable to establish intracerebral infection in mice. 18323 does
- **Potency test took no account of serotype specificity of vaccine or challenge strain**



Intracerebral Mouse Protection

Test (Kendrick Test)

- **Global WHO proficiency study** showed test performed reproducibly (2004)
- Most laboratories obtained valid assays
- Previous collaborative studies conflicting (1981,1997)
- Agreed a difficult assay
- Must see potency test as **PART** of the quality control of whole cell pertussis vaccines
- **May be effective control ONLY when all else in place**



Whole cell pertussis vaccine- quality control (WHO)

- Production strains – phase 1, chosen so that final vaccine contains aggs 1,2,3
- Opacity to control quantity of bacteria (related to toxicity)
- Confirm agglutinogens 1,2,3 in final bulk
- Specific toxicity– Mouse weight gain/others
- Potency by Kendrick test – challenge assay correlated directly with human protection (most data from plain vaccines)

New knowledge

- Concern about alleged serious adverse events following use of whole cell pertussis vaccine led to development of acellular pertussis vaccines
- Considerable investigation of pertussis - the organism, its toxins, molecular biology, pathogenicity, immune mechanisms



Immunity to pertussis

- **Antibodies**
- **Cell mediated immunity** – in mice and humans
- Whole cell vaccine > antibodies to PT, FHA, pertactin, agglutinogens 2,3 (Fims 2,3); contain some active PT
- Acellular vaccines containing same antigens give higher titres than whole cell vaccines; contain very low levels of active PT
- Kendrick test of no use for acellular P vaccines: modified to challenge at 3 weeks
- Presence of active PT influences results.



The Kendrick test

- An effective potency test is available
- When used properly leads to effective whole cell vaccines
- Need always to review situation
- Can a **reliable** simpler alternative be developed and what parameters need to be taken into consideration to do so?

Alternative Potency Tests

- Respiratory challenge model – aerosol/intra-nasal test ?
- Nitric oxide assay ?
- **Serological potency assay ?**
- Original agglutinin assay updated?



Reliable Potency Assay

- Difficulties with acellular pertussis vaccines – no serological correlates of protection
- Rely on showing **consistency** of production
- Consistency only gives adequate assurance if factors tested relevant to clinical protection



Validation

- Correlate to **human protection**: show vaccine behaves poorly in test, behaves poorly in humans
- Direct correlation difficult now
- Correlate with Kendrick test – series of vaccines which FAIL Kendrick test and fail new test : vaccines PASS Kendrick test and pass new test



Alternative Potency Tests

- Still have to be very careful about **what** we are measuring
- Are we covering ALL important parameters which contribute to protection?
- Antibody production, avidity, cell mediated immunity, need for immune responses to required range of antigens?
- **Kendrick test – antibodies AND CMI**
- **Remove Kendrick test lose test for CMI?**



WHO Requirements 1990

- “Mouse protection test still the only recognized test”
- “Further research aimed at establishment of alternative or supplementary tests should be actively encouraged”
- Until an alternative has been shown to be a good indicator of efficacy in humans, the mouse protection test will remain the **ONLY** recognized test for potency of wP vaccines