Report on the ICCVAM / NICEATM International Workshop on Alternatives to the HIST for Acellular Pertussis Vaccines

Richard McFarland, MD, PhD,
FDA

SACATM Meeting
September 23, 2013
National Institute of Environmental Health Sciences
Durham, North Carolina

Etiologic Agent of Whooping Cough (*B. pertussis*)

- Highly contagious disease caused by the bacterium *Bordetella pertussis* and characterized by violent coughing
- Whole cell vaccine introduced in the 1940s
  - Replaced by an acellular vaccine over the last 20 years
- Periodic epidemics every 3 to 5 years and frequent outbreaks
  - During past 5 years, 10,000 to 27,000 cases reported annually in the US

Current *In Vivo* Pertussis Vaccine Safety Testing

- Murine HIST is a key safety test performed to assay for residual active pertussis toxin prior to vaccine release
  - Based on the sensitization to histamine induced by active pertussis toxin
  - Requires large numbers of laboratory animals (mice) that experience unrelieved pain and distress

ICCVAM / NICEATM Vaccine Potency and Safety Testing 2010 Workshop [1]

- Pertussis vaccines were identified as one of the highest priorities for human vaccines for future research, development, and validation efforts because:
  - Many lots are produced annually
  - HIST use large numbers of laboratory animals
  - HIST involves significant unrelieved pain and distress in mice
  - HIST is highly variable often requiring frequent retests

[1]Stokes et al. 2011. The International Workshop on Alternative Methods to Reduce, Refine, and Replace the Use of Animals in Vaccine Potency and Safety Testing: Introduction and Summary. Procedia in Vaccinology 5:1-15.

Alternatives to HIST Vaccine Safety Testing Workshops[1,2]

- Paul Ehrlich Institute workshop (2011) established an International Working Group on Alternatives to HIST for testing alternative *in vitro* methods using standardized acellular pertussis vaccines and pertussis toxin
- Satellite Meeting on Alternative Testing Strategies at 8th Word Congress, Montreal (2011)

[1]Workshop on Animal-Free Detection of PTx in Vaccines - Alternatives to HIST, PEI, Langen, Germany, June 9-10, 2011.
[2]Alternative Safety Testing Strategies for Acellular Pertussis Vaccines (8th World Congress Satellite meeting), Montreal, Canada, August 21, 2011.

Workshop on Alternatives to the Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines

- November 28-29, 2012
- William H. Natcher Conference Center

  National Institutes of Health, Bethesda, MD
- Over 40 scientific experts from 11 countries representing government, academia, and industry
- Plenary and Breakout Group Sessions
- NICEATM coordinated with the International working group on alternatives to HIST
- Workshop report submitted to *Biologicals* for publication

Further information is available at: *http://ntp.niehs.nih.gov/?objectid=857B5F04-F413-8E68-E8640C0A4B788BB4*

2012 ICCVAM / NICEATM Pertussis Workshop Objectives

- Review the usefulness and limitations for alternative *in vitro* test methods proposed to replace the current *in vivo* HIST
- Review *in vitro* protocols and data generated by participants of the International Working Group on Alternatives to HIST
- Discuss application of *in vitro* assays for monitoring consistency of vaccine manufacture as alternatives to the HIST
- Establish framework for international collaboration to validate *in vitro* assay(s) for acellular pertussis vaccine testing
- Identify regulatory acceptance requirements for *in vitro* assays as alternatives to the HIST

2012 ICCVAM / NICEATM Pertussis Workshop: State of the Science

- Biochemical methods: quantitative, robust, fast, and less expensive
  - Only monitor separate functions, not holotoxin activity
- Cell-based assays: more relevant to physiological function of PTx
  - More variable, technically challenging, potentially less sensitive
  - Must be modified for final product testing
- Genetic assays: potentially more relevant system with multiple markers
  - Early stages of development, may not be applicable to routine product release testing

Pertussis Vaccine Adjuvant Desorption Methods: State of the Science

Current Status

- No common desorption method for all vaccines
  - Differences in adjuvant chemistries
  - Depending on selected analysis method, may not be required prior to quantification of residual PTx

Recommendations

- To identify the best desorption method for assay and product, participants recommended the following criteria be established:
  - Desorption must be consistent
  - Minimum sensitivity level must be determined
  - Acceptable and reproducible range of spike recovery must be stated
  - Demonstration that PTx maintains characteristics (e.g., binding activity)
  - Appropriate control(s) must be included: inclusion of PTx-spiked control vaccine in every test was recommended

International Working Group on Alternatives to HIST 2012 Study Outline

- Use of common set of vaccines, pertussis toxin (reference standard), and protocol for spiking
- 12 laboratories
- 7 vaccines from 3 manufacturers (GlaxoSmithKline, Sanofi Pasteur, Statens Serum Institute)
- Biochemical assays
  - Binding assay: used to assess the amount of pertussis toxin/toxoid binding activity to the glycoprotein fetuin
  - Enzymatic assay: monitors residual ADP-ribosylation of the pertussis toxin/toxoid
- Cell-based assays
  - Human cells measuring ATP reduction
  - Rat cells measuring cAMP
  - CHO cell (morphological, cytopathic)

International Working Group on Alternatives to HIST 2012 Study: Data Discussion

- Biochemical Methods
  - ADP-ribosyltransferase (enzyme-HPLC) and carbohydrate-binding ELISA methods
  - Useful for consistency and trend analysis only
- Cell-based Assays
  - Pertussis ATP Test (PAT) and cAMP-PTx Assay
  - Provide a concentration dependent response
  - Further optimization required to increase sensitivity and reduce variability

Recommendation

- Development of specific reagents (rather than reliance on commercial monoclonal Ab kits) would reduce variability

International Working Group on Alternatives to HIST 2012 Study: Data Discussion

- Chinese Hamster Ovary (CHO) Cell Assay
  - Data presented for a modified CHO cell assay
  - Reported detection limit of 1 IU PTx/mL in 48 hr for final vaccine testing
  - Semi-quantitative
    - Morphologic response of CHO cells to PTX may be quantified using Real-Time Cell Analyzer (RTCA)

2012 ICCVAM / NICEATM Pertussis Workshop: Conclusions

- No single method discussed was sufficiently developed for harmonized validation studies at this time
- Single method protocol not applicable to all aP vaccines
  - Differences in inactivation, formulation, and adjuvant
- Goal to identify general assay/testing strategy to accommodate differences between vaccines (e.g., adjuvant types)
- Assays selected for further optimization/testing be robust, sensitive (1-2 IU PTx/mL), reproducible, easy to initiate, and cost effective when compared to HIST

2012 ICCVAM / NICEATM Pertussis Workshop: Conclusions

- Importance of harmonization was recognized for next collaborative study
  - Guidance and basic procedures required, should not be prescriptive
  - Method validation of each product conducted by manufacturers
  - Involvement of regulatory agencies in the planning stages of validation studies would increase likelihood of the acceptance of submissions related to such data
  - Regulatory authorities require that alternative method is:
    - As sensitive as existing animal assay (HIST)
    - Specific in its detection of PTx
    - Flexible enough to cover the universe of vaccines currently tested with HIST

2012 ICCVAM / NICEATM Pertussis Workshop: Recommendations (1)

- Next international collaborative study in 2013
  - Assess use of CHO cell assay for calibration of PTx international reference standard BRP relative to JNIH-5
  - Harmonized CHO cell assay protocol required for study
  - Current CHO assay must be modified to address adjuvant and excipient interference to be successful in vaccine release testing

2012 ICCVAM / NICEATM Pertussis Workshop: Recommendations (2)

- To address adjuvant and excipient interference, participants recommended an initial small collaborative study using both the modified CHO assay and the cAMP-PTx cell based assay
  - cAMP-PTx method be included if sufficient optimization to reduce variability is attained before study initiation
  - 7 laboratories should participate to address the variability of the cAMP-PTx method
  - Fewer representative vaccines should be included in study
    - Include different lots of same vaccine
    - Vaccines with different detoxification methods should be used
  - Spiking concentrations should match lower levels of sensitivity of the HIST assay
  - International Working Group for Alternatives to HIST should provide input and direction for study design

Alternatives to HIST for Pertussis Vaccine Testing: Future Plans

- 2012 ICCVAM / NICEATM Workshop report
  - Submitted to *Biologicals*
  - Expected publication in October, 2013
- Possible Satellite Meeting at World Congress
  - Prague, Czech Republic, 2014
- Next international workshop on Alternatives to HIST
  - London, England 2014
  - Organized by National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs)
    - Support from NICEATM
  - Will review data from proposed collaborative studies

Acknowledgements: Invited Speakers

- **Nicholas Carbonetti, PhD**
  University of Maryland
  USA
- **Christina Bache, PhD**
  PEI
  Germany
- **Amelie Castiaux, IR**
  GSK
  Belgium
- **Erik Hewlett, MD**
  University of Virginia
  USA
- **Marieke Hoonakker, MSc**
  NVL
  The Netherlands
- **Richard Isbrucker, PhD**
  Health Canada
  Canada
- **Juthika Menon, PhD**
  Sanofi Pasteur
  Canada
- **Sue Nelson, PhD**
  Sanofi Pasteur
  Canada
- **Ho-Kyung Oh, PhD**
  KFDA
  South Korea
- **Lev Sirota, PhD**
  FDA
  USA
- **Stefan Vaessen, PhD**
  University of Utrecht
  The Netherlands
- **CT Yuen, PhD**
  NIBSC
  United Kingdom
- **Dorothy Xing, PhD**
  NIBSC
  United Kingdom

Acknowledgements: Pertussis HIST Organizing Committee

Biologics Working Group Members

- **William Stokes, DVM, DACLAM**
  NIEHS
  USA
- **Warren Casey, PhD, DABT**
  NIEHS
  USA
- **Richard McFarland, PhD, MD (Co-Chair)**
  FDA
  USA
- **Marlies Halder, DVM**
  EURL ECVAM
  Italy
- **Richard Isbrucker, PhD**
  Health Canada
  Canada

Ad Hoc Liaisons

- **Juan Arciniega, DSc, (Co-Chair)**
  FDA
  USA
- **Christina Bache, PhD**
  PEI
  Germany
- **Amelie Castiaux,IR**
  GSK
  Belgium

Ad Hoc Liaisons (cont)

- **Jean-Michel Chapsal, PhD**
  Sanofi Pasteur
  France
- **Marieke Hoonakker, MSc**
  NVL
  The Netherlands
- **Sue Nelson, PhD**
  Sanofi Pasteur
  Canada
- **Masaki Ochiai, PhD**
  JaCVAM
  Japan
- **Ho-Kyung Oh, PhD**
  KFDA
  South Korea
- **Lev Sirota, PhD**
  FDA
  USA
- **Dean Smith, PhD**
  Health Canada
  Canada
- **Dorothy Xing, PhD**
  NIBSC
  United Kingdom