VACCINOLOGY – A PUBLIC HEALTH REVOLUTION

M.D. THESIS

"If you asked a public health professional to draw up a top-ten list of the achievements of the past century, he or she would be hard pressed not to rank immunisation first. In short, the vaccine represents the single greatest promise of biomedicine: disease prevention."

A Stern

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Statement of the author

This work contains no material which has been accepted for the award of any other degree* or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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*Manuscript 8
This study was conducted as part of my MPH dissertation but the publication was not submitted as a component of my MPH degree (i.e. was not a requirement of the MPH degree).

Dr Helen Marshall
01 October 2009

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Professor Don Roberton and Professor Philip Ryan and more recently Professor Geoff Davidson have mentored my research career and been active collaborators on many of the studies and manuscripts submitted towards this thesis.

I direct an enthusiastic, dedicated group of research medical officers, research nurses and health scientists, and I could not have written this thesis without their support.

I am grateful for the support of the Vaccinology and Immunology Research Trials Unit (VIRTU) advisory board (Professor Geoff Davidson, Professor Philip Ryan, Associate Professor Peter Richmond and Professor David Isaacs), the members of which have become my research and career advisors and are always available for a frank and honest discussion.

Finally and most importantly I am indebted to my husband David for his patience and understanding, to my children Siobhan, Angus and Imogen for keeping me focused and motivated and to my parents Jack and Rosemary (deceased) who taught me that I can achieve anything.

Abstract

This thesis comprises a collection of publications on new vaccines, vaccine safety and research in implementation of new vaccines into the community to inform public health policy globally. The papers presented outline my research experience in vaccinology which has been conducted in collaboration with a number of national and international colleagues, who are included as coauthors. I have been involved in all aspects of the research including study concepts, conduct, analysis and interpretation of the results and manuscript preparation and publication.

Studies in investigational vaccines outlined in Chapters 1, 2 and 4 were conducted as multicentre studies on the immunogenicity and safety of new vaccines including DTPa-HBV-Hib (diphtheria, tetanus, acellular pertussis, hepatitis B and *Haemophilus influenzae* type b) vaccine, DTPa-HBV-IPV (diphtheria, tetanus, acellular pertussis, hepatitis B and inactivated polio) vaccine, live intranasal attenuated influenza vaccine, Hib-MenCY (*Haemophilus influenzae* type b, *Neisseria meningitidis* serogroups C and Y) vaccine, DTPa-IPV (diphtheria, tetanus, acellular pertussis and inactivated polio) vaccine, PIV3 (parainfluenza virus type 3) vaccine and RSV-PIV3 (respiratory syncytial virus and parainfluenza type 3 virus) vaccine. Many of these vaccines are now licensed in Australia (DTPa-HBV-IPV; "Infanrix-Penta", DTPa-IPV; "InfanrixIPV", HepAB; "Twinrix") with some licensed in other countries (live attenuated influenza vaccine; "FluMist") and others soon to be licensed (Hib-MenCY) or still in clinical development (PIV3, RSV-PIV3).

Licensing of vaccines has been dependent on provision of clinical data of an excellent standard, resulting from clinical studies conducted according to ICH-GCP (International Conference on Harmonisation – Good Clinical Practice) as included in this thesis. Currently, the cost of bringing a vaccine from the laboratory bench to the market is around $1 billion, with much of this cost derived from extensive clinical trial testing undertaken, often directed or influenced by regulatory authorities.

Studies for neonates, young children and adolescents require specific approaches relevant to their needs. Important areas such as recruitment to studies, levels of understanding, needs of families and caregivers, and appropriate care of potentially fearful and tearful participants all need to be addressed carefully and with great skill and support. Issues of assessment of symptoms and potential adverse effects need to be approached differently to those in older independent study participants. Paediatric vaccine clinical trials

can only be successfully conducted with a specialized, experienced and dedicated team of investigators with a wide range of individual skills. Each investigational participant age group requires a specific type of specialist expertise, including skills which may range from venesection of a 2 month old infant (preferably on the first attempt), to blowing bubbles to distract an anxious 4 year old being vaccinated to discussing the study requirement for urine pregnancy testing (as part of study exclusion criteria) to a 12 year old girl. The successful completion of a paediatric vaccine study is dependent on staff that can provide ethical judgement and the required support and consideration for families that are willing to be involved in vaccinology research for the public good. There are only a select group of paediatric vaccinology centres in Australia of which our unit is included. Conducting investigational vaccine trials in Australia has the advantage of providing immunogenicity and safety data in Australian children to Australian regulatory authorities and immunisation expert groups such as the Australian Technical Advisory Group on Immunisation (ATAGI) to inform the optimal immunisation schedule for the Australian population.

Community engagement in and acceptance of new vaccines introduced into the community underpins the success of immunisation programs. Results of social epidemiological studies conducted to examine the introduction of vaccines including varicella, human papillomavirus, and pandemic influenza vaccines are outlined in Chapter 3. Understanding community awareness of vaccines and concerns about vaccine safety is essential for the planning and development of vaccine delivery programs whether delivered through schools, doctors or local government. Results of these studies showed low knowledge of Human Papillomavirus disease and vaccination in the community but acceptance of this cancer preventing strategy if the vaccine was deemed to be safe. Likewise knowledge of pandemic influenza was poor but acceptance of strategies to prevent transmission of infection was assured. Poor uptake of varicella vaccine following licensing was primarily due to poor knowledge about availability of the vaccine and cost of the vaccine prior to funding. Results of these studies have been used to inform public health policy in relation to vaccine delivery to the community.

Continuous review of the Australian National Immunisation Program is required to ensure optimal uptake, timely delivery and acceptability of vaccines. Studies in Chapter 5 outline new strategies to reduce the number of injections required to complete an immunisation schedule while ensuring optimal protection, to reduce the burden of disease in our community.

The work presented in this thesis has supported the timely introduction of new vaccines with knowledge of the community's concerns and acceptance of these vaccines to direct optimal service delivery to achieve high vaccine uptake and reduction in the burden of disease for current and future generations.

Manuscripts submitted for this MD thesis and author contribution

The papers submitted for this thesis comprise original manuscripts, published in international and national peer reviewed journals covering vaccines and public health related topics. My contribution to each study and resulting publication is described for each paper. The main sources from which the information in the papers is derived and the extent to which others have contributed, is described for each publication.

Chapter 1: New Combination Vaccines

1. Marshall H, McIntyre P, Roberton D, Dinan L, Hardt K. Primary and booster immunization with a diphtheria, tetanus, acellular pertussis, hepatitis B (DTPa-HBV) and *Haemophilus influenzae* type b (Hib) vaccine administered separately or together is safe and immunogenic. International Journal of Infectious Diseases. 2009 (In press).

Impact Factor: 2.210 Ranking amongst infectious disease journals: 36/57 Citations: 0

This Phase 3 study was conducted in infants from 2 months of age to determine whether a combined diphtheria, tetanus, acellular pertussis, hepatitis B and *Haemophilus influenzae* type b vaccine (DTPa-HBV-Hib) is as immunogenic as DTPa-HBV and Hib given separately. The study was conceived by our research group in collaboration with the National Centre for Immunisation Research and Surveillance (Professor Peter McIntyre) and with GlaxoSmithKline. It was supervised by Professor Don Roberton and conducted by me with the support of research staff. I interpreted the results and wrote the manuscript, with the help of co-authors.

2. **Marshall H,** Nolan T, Roberton D, Richmond P, Lambert S, Jacquet J-M, Schuerman L. A comparison of booster immunisation with a combination DTPa-IPV vaccine or DTPa plus IPV in separate injections, when co-administered with MMR at age 4-6 years. Vaccine. 2006;*24:6120-6128.*

Impact Factor: 3.616 Ranking amongst immunology journals: 36/128 (top in Vaccinology) Citations: 7

This Phase 3b multicentre, non-inferiority study was conducted in children 4-6 years to compare separate administration of diphtheria, tetanus, acellular pertussis (DTPa) vaccine and inactivated polio (IPV) vaccine to a combined DTPaIPV vaccine when administered to school age children, in order to reduce the number of injections required to provide adequate protection against these four infectious diseases. The study was designed by GlaxoSmithKline with input from Investigators. I wrote the manuscript describing the study results, with input from co-authors.

3. Nolan T, Lambert S, Roberton D, **Marshall H,** Richmond P, Streeton C. DTPa-HBV-IPV vaccine for primary vaccination of infants. Journal of Paediatrics and Child Health. 2007;43:576-581.

Impact Factor: 1.4 Ranking amongst paediatric journals: 59/94 Citations: 0

This Phase 2 study assessed the immunogenicity and safety of a combination diphtheria, tetanus, acellular pertussis, hepatitis B and inactivated polio (DTPa-HBV-IPV) vaccine for use in infants from 2 months of age to reduce the number of needles required in infancy to provide adequate protection for infants, against five infectious diseases. The study was designed byGlaxoSmithKline and conducted at three centres in Australia. I supervised the study at our site and was involved in the study conduct and contributed to the study design. The manuscript was written by all co-authors.

4. Nolan T, Lambert S, Roberton D, **Marshall H**, Richmond P, Streeton C, Poolman J, Boutriau D. A novel combined *Haemophilus influenzae* type-b-*Neisseria meningitidis* serogroups C and Y-tetanus-toxiod conjugate vaccine is immunogenic and induces immune memory when co-administered with DTPa-HBV-IPV and conjugate pneumococcal vaccines in infants. Vaccine. 2007;25:8487-8499.

Impact Factor: 3.616 Ranking amongst immunology journals: 36/128 (top in Vaccinology) Citations: 20

A combined "meningitis" vaccine to provide protection against common causes of meningitis including *Haemophilus influenzae* type b and *Neisseria meningitidis* serogroups C and Y was investigated at several clinical trial centres in Australia, including our research unit. This Phase 2 study was designed by GlaxoSmithKline and conducted at 3 centres in Australia in infants from 2 months of age. I supervised the study at our site and contributed significantly to the paper describing the study results.

Chapter 2: New Respiratory Virus Vaccines

5. Nolan T, Bernstein D, Block S, Hilty M, Keyserling H, Marchant C, **Marshall H**, Richmond P, Yogev R, Cordova J, Cho I, Mendelman P and for the LAIV Study Group. Safety and Immunogenicity of Concurrent Live Attenuated Influenza Vaccine With Measles-Mumps-Rubella and Varicella Vaccines in Infants 12 to 15 Months of Age. Pediatrics 2008;121:508-516.

Impact Factor: 5.665 Ranking amongst paediatric journals: 2/94 Citations: 9

This Phase 3 multicentre, international study was conducted to determine the safety and immunogenicity of an intranasal, live attenuated vaccine for prevention of influenza when given concurrently with measles, mumps, rubella vaccine to infants 12-15 months of age. I conducted the study, with support from research staff and the paper describing the results was written by all co-authors.

6. Belshe RB, Newman FK, Tsai TF, Karron RA, Reisinger K, Roberton D, **Marshall H**, Schwartz R, King J, Henderson FW, Rodriguez W, Severs JM, Wright PF, Keyserling H, Weinberg GA, Bromberg K, Loh R, Sly P, McIntyre P, Ziegler JB, Hackell J, Deatly A, Georgiu A, Paschalis M, Wu SL, Tatem JM, Murphy B, Anderson E. Phase 2 Evaluation of Parainfluenza Type 3 Cold Passage Mutant 45 Live Attenuated Vaccine in Healthy Children 6–18 Months Old. Journal of Infectious Diseases. 2004;189:462-470.

Impact Factor: 5.865 Ranking amongst infectious disease journals: 4/57 Citations: 21

This Phase 2 experimental study examined the safety and immunogenicity of an intranasal live attenuated parainfluenza virus type 3 (PIV3) vaccine administered to healthy children 6-18 months of age. The protocol was designed by Wyeth-Lederle vaccines with input from co-investigators. I conducted the study, supervised by Professor Don Roberton, and with the help of research staff. The study results are presented in this paper with all co-authors contributing to interpretation of the results and writing of the manuscript.

7. Belshe RB, Newman FK, Anderson EL, Wright PF, Karron RA, Tollefson S, Henderson FW, Meissner C, Madhi S, Roberton D, **Marshall H**, Loh R, Sly P, Murphy B, Tatem JM, Randolph V, Hackell J, Gruber W, Tsai TF. Evaluation of Combined Live, Attenuated Respiratory Syncytial Virus and Parainfluenza 3 Virus Vaccines in Infants and Young Children. Journal of Infectious Diseases. 2004;190:2096-2103.

Impact Factor: 5.865 Ranking amongst infectious disease journals: 4/57 Citations: 23

This multicentre Phase 1 study examined the safety and immunogenicity of a live attenuated intranasal combined respiratory syncytial virus and parainfluenza virus (RSVPIV3) vaccine in children 6-18 months of age. The protocol was designed by Wyeth-Lederle vaccines with input from co-investigators. I conducted this study, with the help of other research staff. The results are presented in this paper which was contributed to by all co-authors.

Chapter 3: Community and Immunisation Provider Acceptance of New Vaccines

8. Marshall H, Ryan P, Roberton D. Uptake of varicella vaccine – a cross sectional survey of parental attitudes to nationally recommended but unfunded varicella immunisation. Vaccine. 2005;23:5389-97.

Impact Factor: 3.616 Ranking amongst immunology journals: 36/128 (top in vaccinology) Citations: 12

The aim of this study was to assess the uptake of varicella vaccine in South Australian children under circumstances where varicella immunisation is recommended but is not funded by Government. The study examined the main reasons that determined a parent's decision whether or not to have their child immunised with varicella vaccine. The study concept was mine and I designed and conducted the study, including the statistical analysis, performed with the help of Professor Philip Ryan, as part of my dissertation for my Master in Public Health degree. However, the paper was not written or presented as part of the dissertation and, therefore, is appropriate to include in this thesis. I wrote the paper, and all co-authors contributed.

9. **Marshall H,** Ryan P, Roberton D, Beilby J. Varicella immunisation practice: Implications for provision of a recommended, non-funded vaccine. Journal of Paediatrics and Child Health. 2009;45:297-303.

Impact Factor: 1.4 Ranking amongst paediatric journals:59/94 Citations: 2

Introduction of a new vaccine without providing government funding has been shown to result in low uptake of the vaccine. This study assessed the factors influencing the use of a recommended but not funded vaccine by general practitioners. The study concept and design was my own and I conducted the study, with support of other research staff. I performed the statistical analysis and I was the principal author of the manuscript with input from co-authors.

10. **Marshall H**, Ryan P, Roberton D, Baghurst P. A cross-sectional survey to assess community attitudes to introduction of Human Papillomavirus vaccine. Australian and New Zealand Journal of Public Health. 2007;31(3):235-242.

Impact Factor: 1.793 Ranking amongst public health journals: 73/122 Citations: 36

This study was conducted to assess community understanding and acceptance of human papillomavirus (HPV) vaccine, introduced to prevent cervical cancer in women. The study preceded the implementation of the immunisation program in Australia. I performed the study using grant money I received as the inaugural recipient of the Post Masters Public Health Education and Research Trust award. The study concept was mine and I conducted the study, performed the statistical analysis (with help from Professor Philip Ryan) and wrote the manuscript, with input from co-authors.

11. **Marshall H**, Ryan P, Roberton D, Street J, Watson M. Pandemic Influenza and Community Preparedness. American Journal of Public Health. 2009;99:S365-71.

Impact Factor: 4.984 Ranking amongst public health journals: 9/122 Citations: 2

The aim of this study was to assess community and parental knowledge of and attitudes toward the threat of pandemic influenza and acceptance of vaccination with novel pandemic influenza vaccines to provide protection against this global threat. The concept of the study was mine and I devised the study design and completed the statistical analysis. I was the principal writer of the manuscript with input from co-authors.

12. Isaacs D, Kilham H, **Marshall H.** Should routine childhood immunizations be compulsory? Journal of Paediatrics and Child Health. 2004;40:392-396.

Impact Factor: 1.4 Ranking amongst paediatric journals: 59/94 Citations: 4

In Australia, immunisation is not compulsory. However, mandatory immunisation, particularly for health care workers who may transfer infection to patients, is being considered in some states in Australia. This review paper was written to outline and discuss the ethical issues associated with compulsory immunisation. Although Professor David Isaacs was first author, I contributed significantly to ideas in the paper, based on a public health ethics course I completed during my Master in Public Health degree.

Chapter 4: Vaccine Safety

13. Jacquet JM, Begue P, Grimprel E, Reinert P, Sandbu S, Silverdal SA, Faldella G, Nolan T, Lambert S, Richmond P, **Marshall H,** Roberton D, Schuerman L. Safety and immunogenicity of a combined DTPa-IPV vaccine administered as a booster from 4 years of age: a review. Vaccine. 2006;24:2440-2448.

Impact Factor: 3.616 Ranking amongst immunology journals: 36/128 (top in Vaccinology) Citations: 11

In this review paper we assessed the safety results of all clinical trials conducted with the combined DTPa-IPV vaccine, including the study described above (combination vaccines Paper no. 2). Booster doses of DTPa-containing vaccines have been associated with large local reactions, with an incidence of 2-22% depending on measurement criteria. This paper reviews the accumulated data on safety and immunogenicity of the DTPaIPV vaccine. The manuscript was written by all co-authors who had contributed to the clinical trials.

14. **Marshall H,** Gold M, Roberton D, Gent R, Quinn P, Piotto L, Clarke M. Ultrasound Examination of Extensive Limb Swelling Reactions After Diphtheria-Tetanus-Acellular Pertussis or Reduced-Antigen Content Diphtheria-Tetanus-Acellular Pertussis Immunization in Pre-school-Aged Children. Pediatrics. 2006;118(4):1501-1509.

Impact Factor: 5.665 Ranking amongst paediatric journals: 2/94 Citations: 8

Through effective immunisation programs, the mortality associated with many infectious diseases has been reduced significantly or eliminated. For some vaccines, this has led to a higher reported incidence of adverse events following vaccination than the current incidence of the disease being prevented. As vaccine preventable infectious diseases are so well controlled in the community, public concern related to adverse events following vaccination rises and the public forgets the risk of the vaccine preventable disease. Research in vaccine safety should continue to be a priority to maintain confidence in the community when new vaccines are introduced into the community.

As mentioned in the previous paper, large local reactions have been associated with booster DTPa-containing vaccines. This clinical study evaluated extensive swelling reactions following booster diphtheria-tetanus-acellular pertussis vaccination at school age. Ultrasound was used to assess extent of the swelling in both subcutaneous and muscle tissue. I conceived the study with Professor Don Roberton and I designed the study. Ultrasound examinations were conducted by the paediatric ultrasonographers (Mr R Gent and Mr Leno Piotto, Paediatric Radiology, Women's and Children's Hospital) under my supervision. I conducted the statistical analysis and wrote the paper, with support from co-authors.

Chapter 5: New Vaccine Schedules

15. Wood N, McIntyre P, **Marshall H**, Roberton D. Acellular pertussis vaccine at birth and one month induces antibody responses by two months of age. Pediatric Infectious Disease Journal. (Accepted for publication, August 4, 2009).

Impact Factor: 3.378 Ranking amongst infectious disease journals: 20/57 Citations: 2

The highest mortality rates from pertussis occur in infants less than 6 months of age who may not have received any or have received a reduced number of vaccines required to provide protection against pertussis. Both Professor McIntyre and I have had an interest in pertussis, which continues to be a poorly controlled infection causing epidemics and deaths in Australian children and millions of deaths in the

developing world. This study aimed to investigate whether giving pertussis vaccine to infants at birth would provide earlier protection for these vulnerable infants. The study was conducted at The National Centre for Immunisation Research and Surveillance (Professor P McIntyre) and the Paediatric Trials Unit at the Women's and Children's Hospital (D Roberton, H Marshall). The concept protocol was designed by Professor McIntyre, with final protocol designed by all co-authors. I supervised the study and was involved in study conduct at the Adelaide site and all co-authors contributed to interpretation of the results and preparation of the manuscript.

16. White OJ, Rowe J, Richmond P, **Marshall H**, McIntyre P, Wood N, Holt PG. Th2-polarisation of cellular immune memory to neonatal pertussis vaccination. Vaccine. (Accepted for publication, August 5, 2009)

Impact Factor: 3.616 Ranking amongst immunology journals: 36/128 (top in vaccinology) Citations: 0

This study explored the cell mediated immune (CMI) responses in infants enrolled in the above study (Paper 15), comparing CMI responses between infants receiving pertussis vaccine at birth and those receiving the routine immunisation schedule (Hepatitis B vaccine only at birth). P Holt and P McIntyre designed the study with laboratory testing completed by P Holt and O White. The interpretation of the results and writing of the manuscript was contributed to by all co-authors.

17. Roberton D, Marshall H, Dinan L, Boros C, Gold M. Developmental immunology and vaccines. Expert Review of Vaccines. 2004;3(4):343-347.

Impact Factor: 4.214 Ranking amongst immunology journals: 25/128 Citations: 3

Preterm infants are at increased risk of early onset of infectious diseases due to compromised immunity. Therefore vaccine schedules for infants born prematurely are different than for term infants, including the administration of additional booster vaccines. This review paper discusses the additional vaccine requirements for premature infants and recommendations to provide adequate protection for these vulnerable infants. I was the principal writer of the paper, with input from co-authors.

18. Roberton D, **Marshall H**, Nolan T, Sokal E, Diez-Domingo J, Flodmark C-E, Rombo L, Lewald G, de la Flor J, Casanovas J, Verdaguer J, Mares J, Van Esso D, Dieussaert I, Stoffel M. Reactogenicity and immunogenicity profile of a two-dose combined Hepatitis A and B vaccine in 1-11 year old children. Vaccine 2005;*23:5099-5105.*

Impact Factor: 3.616 Ranking amongst immunology journals: 36/128 (top in vaccinology) Citations: 7

This Phase 3 multicentre study was conducted in children aged 1-11 years of age to establish whether a two dose Hepatitis A and Hepatitis B combination vaccine was at least as good as a three dose schedule in relation to safety and immunogenicity with the potential to reduce the number of injections required for adequate protection against both Hepatitis A and Hepatitis B. Our centre was the main recruiting centre for this study. The protocol was designed with input from all Investigators. I supervised the study and the paper was written by all Co-Investigators. Participants in this study have continued in a long term follow-up study, recently completed, which I also supervised. I am currently writing a further manuscript outlining the long term immunogenicity results, with input from co-authors.

Introduction

The gasping breath and post-tussive vomiting of an infant, the iron lungs and braces required by children paralysed with polio and the devastating birth defects following maternal rubella infection are now mostly a scourge of the past due to the success of vaccination. Over the past 200 years, since the time of Edward Jenner, vaccination has controlled a dozen major diseases including smallpox, diphtheria, tetanus, yellow fever, pertussis, *Hamophilus influenzae* type b, invasive pneumococcal disease, poliomyelitis, measles, mumps, rubella and rotavirus gastroenteritis. Apart from individual protection, vaccination also protects others in the community by increasing the level of immunity and minimizing the spread of infection. Even in the developing world where infectious diseases cause high mortality, global funding agencies such as the Global Alliance for Vaccines and Immunisation (GAVI) and the World Health Organization (WHO) in partnership with pharmaceutical companies such as GlaxoSmithKline and Merck are making vaccines more readily available.

However opportunities remain to improve immunisation policy to eradicate infections that still occur in epidemics such as pertussis and seasonal influenza and in pandemics such as novel influenza strains. The recent H1N1 pandemic emphasizes the need for continuing innovative technologies to control disease by vaccination in addition to other public health strategies.

The development of new vaccines such as meningococcal, human papillomavirus and respiratory virus vaccines, to address new indications such as cervical cancer and respiratory infections, has only been achievable due to the development of new technologies.

Improvements in vaccine delivery, including the use of combination vaccines to reduce the number of needles administered to children is likely to enhance acceptability of new vaccines in the community. In addition, new techniques for vaccine administration, including nasal drop or nasal spray delivery are likely to increase compliance and improved immune response with induction of both mucosal and humoral immunity.

However, the most pressing problem in the 21st century is the provision of modern vaccines to the poorest parts of the world with the highest burden of disease.

The recent past has shown a growing focus on vaccine safety. Given that vaccines are administered mainly to healthy children and adults in many countries, rare but serious illnesses that would have occurred anyway will coincidentally follow vaccination. Understanding adverse events by acquiring valid scientific data and informing the community in a transparent and timely fashion is essential to avoid erosion of community confidence in vaccination.

Vaccinology is an evolving discipline requiring extensive research into epidemiology of disease, clinical trials of new vaccines and social epidemiology to ensure the safety and effectiveness of new vaccines with optimal intake once introduced into the community.

This thesis describes my 12 years of research in vaccinology and the contribution I have made to this *public health revolution* with the help of many colleagues.

Chapter 1: New Combination Vaccines

New technologies have expanded the number of infectious diseases we are now able to prevent through vaccination. However there is likely to be a limit to the number of injections parents find acceptable to be administered to their children at one vaccination encounter. The use of combination vaccines provides a practical way of providing the greatest protection with timely vaccination coverage for the youngest, most vulnerable infants.

The advantage of combining antigens to protect against a multitude of infections in one vaccine has prompted further research into both the pharmaceutical and immunological interactions between vaccines. However antigens cannot be combined indiscriminately as each combination may affect the immunogenicity of the individual components. Little is known about the immunological interactions caused by combinations of vaccine antigens. Chemical incompatibility or immunologic interference may result in a vaccine that is immunologically inferior to separate administration of the vaccine components. Factors such as pH of the medium, the presence and type of adjuvant and the type of preservative used may influence the immunogenicity of the different components of the combination vaccine.

Therefore it is essential that every new combination vaccine be tested in clinical trials to establish whether the new combination vaccine provides at least as good immunogenicity as separate vaccines containing the same antigens. As outlined in this chapter, *Haemophilus influenzae* type b (Hib) vaccines can show reduced immunogenicity when combined with diphtheria, tetanus, acellular pertussis (DTPa) vaccines (Paper 1) but increased immunogenicity when added to other conjugate vaccines (Paper 4). Not only is it important to compare the amount of antibody but also the quality or functionality of the antibodies produced. In addition, consistency in immunogenic response must be demonstrated amongst vaccine lots prior to a vaccine being licensed.

Notably, large safety studies are also required to ensure that new combination vaccines do not cause more reactogenicity than separately administered vaccines.

Once the combination vaccine has been trialed and found to be at least as immunogenic and safe as separate administration of the vaccine components, the impact on the immunogenicity and reactogenicity of other co-administered vaccines needs to be evaluated.

Infant vaccines are given from two months of age to provide protection as early as possible against infections known to cause morbidity or sequelae in this vulnerable age group. Combination DTPa vaccines have been used for the past 15 years in Australia. The obvious next step is to combine other newer antigens with DTPa to be given to infants in one injection.

The studies presented in Chapter 1 of this thesis provide valuable insights into clinical research with new combination vaccines, several of which are now licensed in Australia or overseas.

Immunological Interference in combination vaccines containing *Haemophilus influenzae* type b

1. Marshall H, McIntyre P, Roberton D, Dinan L, Hardt K. Primary and booster immunization with a diphtheria, tetanus, acellular pertussis, hepatitis B (DTPa-HBV) and *Haemophilus influenzae* type b (Hib) vaccine administered separately or together is safe and immunogenic. International Journal of Infectious Diseases. 2009 (In press).

Immunological interference is known to occur from studies of combination vaccines including *Haemophilus influenzae* type b. Little is understood about the mechanism of interference giving rise to marked depression of responses to Hib conjugates in combination vaccines also containing diphtheria, tetanus, acellular pertussis antigens.

In Paper 1 the immunogenicity and safety of a combined diphtheria, tetanus and acellular pertussis, hepatitis B and *Haemophilus influenzae type b* (DTPa-HepB-Hib) vaccine was compared with separate administration of diphtheria, tetanus and acellular pertussis hepatitis B (DTPa-HepB) and *Haemophilus influenzae type b (Hib)* for primary vaccination in infants. A follow-up study investigated the immunogenicity and safety of booster vaccination with DTPa-HBV-Hib as a single injection given in the second year of life.

Primary immunisation with the combined vaccine resulted in high levels of seroprotection, (94%) against the Hib PRP antigen and 97% seroprotection for separate injections. For hepatitis B, no difference was

observed between the combined or separate methods of vaccine administration. However the proportion of subjects with anti-PRP antibody levels consistent with long term protection (≥1.0 µg/ml), and anti-PRP antibody geometric mean concentration (GMC), was higher in the group that received separate injections. Importantly, Hib antibody concentrations of > 1.0 µg/ml have been shown to be associated with long-term protection. As mentioned above, the combined administration of Hib tetanus-conjugated vaccines with DTPa-based vaccines can reduce the level of circulating antibodies to Hib PRP compared to separate administration of the Hib vaccine. However, it has also been shown that the functional nature of the antibodies against Hib produced by combined DTPa-Hib vaccines is the same as those induced by separate injections, and that immunological memory is induced. Although a lower Hib GMC was measured in children who received the combination vaccine, this difference is unlikely to have clinical significance. This is supported by epidemiological data that show a decrease in Hib disease in countries using combination DTPa-Hib vaccines in their immunisation program.

In this study, a dose of plain PRP was given to infants at 12 months of age as a "pseudo challenge" mimicking Hib infection, to demonstrate effective priming and immunological memory in children who developed a lower antibody response to Hib. The anamnestic response observed following plain PRP challenge and the booster DTPa-HBV-Hib dose confirmed results from other studies with DTPa-Hib combination vaccines showing induction of immune memory.

This response to PRP challenge, at an age where no significant response to the polysaccharide is expected, is indicative of immunological memory and confirms the findings of other studies.

The importance of a booster dose of Hib conjugate vaccine in achieving effective and long-term immunity is well recognised. In Germany, missing the recommended booster dose was associated with an increase in Hib disease. Immunity after primary vaccination without booster was shown to wane over time in the United Kingdom, with a fall in vaccine effectiveness to 37.3% two years post vaccination. Concerns about the efficacy of Hib in DTPa-Hib combination vaccines have been negated by epidemiological data showing that when these vaccines have been included in routine infant schedules they have been highly successful in prevention of Hib disease.

In terms of other vaccine antigens, the proportion of subjects who developed seroprotective antibody concentrations against diphtheria, tetanus and hepatitis B or a vaccine response to pertussis antigens after the booster dose was high, and robust increases in antibody concentrations were observed regardless of the vaccine administered for the primary vaccination course.

In relation to safety, combining the DTPa-HBV and Hib vaccines did not result in increased reactogenicity for either primary or booster vaccination. In this study, the combined DTPa-HBV/Hib booster vaccination was found to be safe. DTPa-HBV and Hib vaccines were shown to be safe and immunogenic whether administered as a single injection or as separate injections for primary and booster vaccination of infants.

This study adds to the literature on combination vaccines by examining further the immunogenicity of combination vaccines containing Hib and demonstrating an anamnestic response to a primary course of Hib vaccine in children identified as low responders to Hib.

Combination vaccines and co-administered vaccines

2. **Marshall H,** Nolan T, Roberton D, Richmond P, Lambert S, Jacquet J-M, Schuerman L. A comparison of booster immunisation with a combination DTPa-IPV vaccine or DTPa plus IPV in separate injections, when co-administered with MMR at age 4-6 years. Vaccine. 2006; 24: 6120-6128.

The World Health Organisation has implemented polio immunisation programs globally to reduce and hopefully eliminate polio in both the developed and developing world. In countries such as Australia and the United States of America (USA), where oral (sabin) live attenuated polio vaccine (OPV) has been given routinely as part of infant immunisation programs for the past 40 years, wild type polio has essentially been eliminated. However OPV is associated with a rare side effect – vaccine associated paralytic polio (VAPP).

Inactivated polio vaccines (IPV) were used in the 1950s before the development of live attenuated OPV and have been used exclusively for polio control by some countries since that time. Use of IPV has the advantage of eliminating the small (1 case in 2.4 million doses) but significant risk of VAPP. In the USA where 8-10 cases of VAPP were reported each year, IPV was introduced in 1997 to ensure no further VAPP occurred. Australia introduced IPV into the national immunisation program in 2005 in the form of a

combination vaccine to avoid separate administration of IPV when children are already receiving three injections at many immunisation encounters. However prior to introduction of a combination vaccine into the routine immunisation schedule the vaccine must be demonstrated to show no interference with other co-administered vaccines. Paper 2 describes a study conducted to assess the safety and immunogenicity of a combination diphtheria, tetanus, acellular pertussis and inactivated polio (DTPa-IPV) vaccine compared to separate administration of DTPa and IPV co-administered with measles, mumps, rubella (MMR) vaccine as a pre-school booster.

Comparisons of the immune responses against all antigen components administered in combined and separate administration, confirmed no difference in immunogenicity.

The immune responses to MMR antigens administered concomitantly were similar whether DTPaIPV or DTPa +IPV was administered. This study provided immunogenicity and safety data for co-administration of a pre-school booster MMR vaccine with DTPa-IPV which had not been available previously. Reactogenicity events and adverse events to MMR vaccine were similar in both groups and occurred in this study in similar or reduced frequency to other literature reports.

It is expected that increased use of IPV in the form of combination vaccines will reduce the frequency of VAPP due to oral live poliovirus vaccine. The introduction of IPV containing combination vaccines has not only resulted in fewer injections for infants and children but also the opportunity to provide safer vaccines for children.

Data from this study were included in the file submitted to the Therapeutic Goods administration (TGA) for licensure of this vaccine in Australia. The vaccine, "InfanrixIPV" is currently used in the National Immunisation Program (NIP) in Australia and in other countries such as the US (Kinrix™).

Combination vaccine – the importance of consistency

3. Nolan T, Lambert S, Roberton D, **Marshall H,** Richmond P, Streeton C. DTPa-HBV-IPV vaccine for primary vaccination of infants. Journal of Paediatrics and Child Health. 2007;43:576-81.

The complications inherent in the development of combination vaccines should not be underestimated. Apart from the technological advances involved, there are increasingly rigorous regulatory requirements

that mandate particular conditions products must meet. Today's regulatory environment is much more complex than that which existed when older combination vaccines were originally licensed.

The development of a combination vaccine containing a complex mixture of components, some which may be themselves combinations of antigens (eg IPV or Pertussis) requires that the product must be manufactured in a consistent way. In addition, different regulatory authorities in different countries have different requirements for licensing of vaccines.

Paper 3 describes the immunogenicity and safety results of a combination diphtheria, tetanus, acellular pertussis, hepatitis B and inactivated polio (DTPa-HBV-IPV) vaccine in Australian children. The immune response to and reactogenicity of the combined vaccine was previously demonstrated to be similar to that of separate administration of the component vaccines. Consistency of the product was measured by comparing the immune response in children receiving vaccine produced from small manufacturing lots to vaccine produced from large manufacturing lots.

In this study we were able to demonstrate that the DTPa-HBV-IPV vaccine, including vaccine manufactured by the larger scale IPV manufacturing process, was highly immunogenic and safe in Australian children, with the vast majority of subjects developing seroprotective antibody concentrations against diphtheria, tetanus, hepatitis B and polio, and a vaccine response to pertussis antigens after primary vaccination. Our study showed that DTPa-HBV-IPV had a good safety profile and was well tolerated when administered to Australian children.

Local reactogenicity in the combined DTPa-HBV-IPV group (including the small and large IPV manufacturing process cohorts) was similar to that of the licensed Hib vaccine given in the opposite limb during the same study visits, an observation made previously in German infants when given at 3-4-5 months of age. Safety after completion of the primary vaccination course was assessed for an extended period with no evidence of long-term adverse effects related to vaccination.

The data from this study were included in the file for licensure with the TGA and Federal Drug Administration (FDA). The DTPa-HBV-IPV vaccine is now licensed in Australia under the trade name

"Infanrix Penta" and is used in the immunisation program in the Northern Territory and in the US is licensed under the trade name "Pediarix".

Combination "Meningitis" vaccines

4. Nolan T, Lambert S, Roberton D, **Marshall H**, Richmond P, Streeton C, Poolman J, Boutriau D. A novel combined *Haemophilus influenzae* type-b-*Neisseria meningitidis* serogroups C and Y-tetanus-toxiod conjugate vaccine is immunogenic and induces immune memory when co-administered with DTPa-HBV-IPV and conjugate pneumococcal vaccines in infants. Vaccine. 2007;25:8487-8499.

Haemophilus influenzae type b, pneumococcus and meningococcus are the commonest causes of bacterial meningitis in children. Ideally a combination vaccine to provide protection against all three bacteria would simplify the vaccination schedule. Pneumococcal vaccines currently in use are conjugate vaccines providing protection against seven pneumococcal serotypes causing invasive disease in children. A recently licensed pneumococcal conjugate vaccine, "Synflorix", will provide protection against 10 different serotypes.

Vaccines to prevent meningitis caused by Hib and *Neisseria meningitidis* are now being developed. There are five different meningococcal serogroups (A, B, C, W, Y) that cause invasive disease in humans. In Australia, prior to the introduction of a meningococcal C vaccine program, approximately 33% of cases were caused by serogroup C and 66% by serogroup B. This program has been so successful that cases due to serogroup C are now rare with 90% of cases due to serogroup B and up to 7% of cases due to serogroups W or Y. Internationally, serogroup Y is a significant cause of meningitis. During the last decade in the US, the proportion of meningococcal disease due to serogroup Y rose from 2% in 1990-1992 to 39% in the period between 1996 and 2001. In the same period, serogroup C accounted for 31% of cases overall, making serogroups C and Y together the cause of approximately two out of three cases of meningococcal disease in the US.

By combining a Meningococcal CY conjugate vaccine with an existing Hib conjugate vaccine infants would receive additional protection without extra injections. This novel Hib-MenCY-TT conjugate vaccine is the first investigational vaccine that combines the Hib antigen with conjugated MenC and MenY antigens in

order to extend protection of infants against further serogroups contributing to meningococcal disease in children.

In Paper 4, the evaluation of a novel combined *Hib-N. meningitidis* serogroup C and Y vaccine conjugated to tetanus toxoid (Hib-MenCY-TT) for the primary vaccination of infants is described. Hib-MenCY-TT in three different dosages was administered concomitantly with routinely administered, commercially available vaccines. This results of this study showed that the Hib and MenC components of the Hib-MenCY-TT vaccine were as immunogenic as currently licensed monovalent Hib and Men C vaccines. The MenY component resulted in bactericidal antibodies in at least 98% of vaccinated infants.

These results demonstrated that the Hib-MenCY-TT vaccine is likely to provide protection against disease due to Hib and *N. meningitidis* type C and type Y. Antibody persistence after primary vaccination was assessed at 11 to 14 months of age. At the same time immune memory (or an anamnestic response) was assessed by immunisation with a reduced dosage (10 ug) of plain polysaccharide (to mimic meningococcal infection).

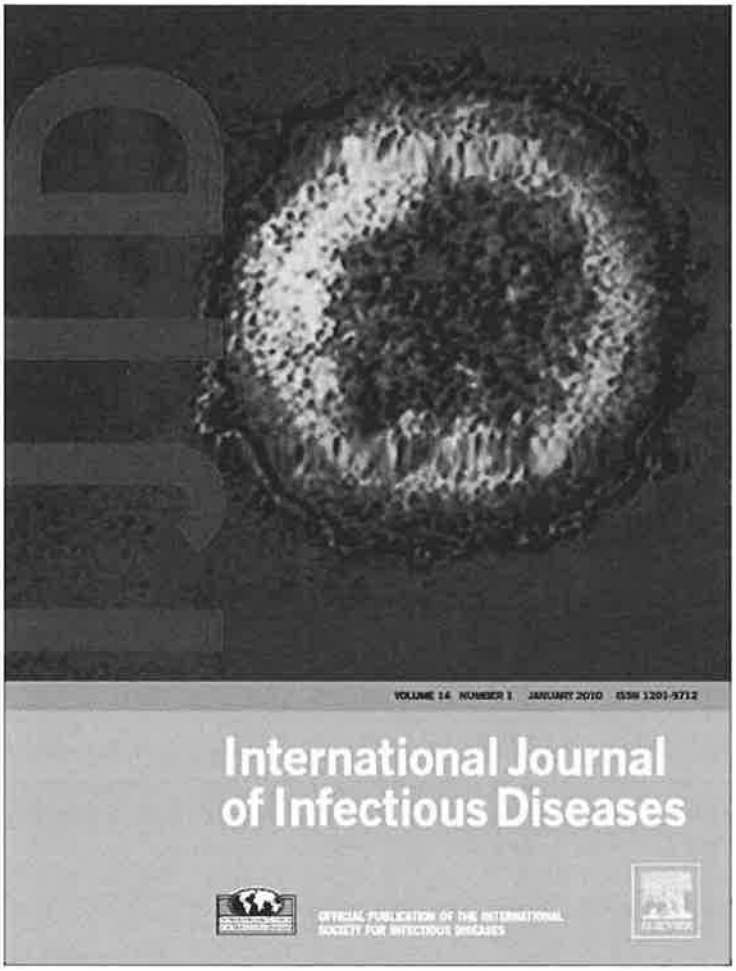
We observed significantly higher levels of MenC antibody persistence in some Hib-MenCY-TT groups (2.5/5/5 and 5/10/10), who had lower post-primary bactericidal MenC antibody levels compared to the licensed MenC vaccine. In the Hib-MenCY-TT groups (2.5/5/5 and 5/10/10), a higher proportion of subjects achieved protective bactericidal MenC titres ≥1:128 after polysaccharide than subjects who received the licensed Men C vaccine. Although interference with tetanus toxoid (TT) based polysaccharide vaccines has previously been shown, we did not observe this in the current study with Hib-MenCY-TT. It is likely that not only the amount of TT plays a role, but that there are critical factors related to the number of polysaccharides that use TT as a carrier.

The three dose levels of Hib-MenCY-TT combination vaccine tested contained lower amounts of Hib PRP than currently licensed Hib vaccines. The ability of vaccines containing reduced amounts of Hib PRP-TT to induce priming and immune memory that is equivalent to licensed products has now been well documented both for monovalent and combined Hib vaccines containing reduced Hib PRP-TT. The tetanus toxoid carrier protein used to conjugate the two meningococcal components of the vaccine may also have enhanced the immunogenicity to the Hib vaccine. There was a higher response in the Hib-MenCY-TT

2.5/5/5 and 5/10/10 groups compared with the licensed Hib vaccine, both in terms of the proportion of subjects who reached the 1.0µg/mL cut-off (long term protection) and antibody GMCs after the polysaccharide challenge. The results of this study lead to the selection of the dose of Hib-MenCY-TT (2.5/5/5) that would be used for Phase III clinical trials and eventually the licensed product.

In the countries where a substantial proportion of meningococcal disease is caused by serogroup Y, a bivalent CY-vaccine is needed to have any substantial impact on meningococcal disease control. The present study demonstrated that MenC and MenY conjugated to TT can be successfully combined with Hib conjugate vaccine containing a reduced amount of PRP without compromise in the immune response or reactogenicity profile of any component, and thereby avoiding additional injections in the already crowded immunisation schedule. The results of this study suggest that Hib-MenCY-TT 2.5/5/5 vaccine can be administered in a 2-4-6 month schedule with other recommended vaccines, without immune interference. Subjects primed with the Hib-MenCY-TT 2.5/5/5 formulation showed consistently higher bactericidal activity and antibody responses to MenC and PRP after polysaccharide challenge, compared to those primed with commercially available monovalent Hib and MenC vaccines. In terms of the MenC response, better persistence from the primary response till the pre-booster time-point was also observed. The MenY component of the novel vaccine was also shown to be immunogenic and induces successful priming with a robust immune memory response. The safety profile in the Hib-MenCY-TT groups was at least as good when compared to the licensed Hib and Men C vaccines.

The results of this study were presented at the annual meeting of the Pediatric Academic Societies (PAS), San Francisco, USA, April 29 - May 2, 2006 and the 45th Annual meeting of the Infectious Diseases Society of America (IDSA) San Diego, California, October 4-7, 2007.



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Primary and booster immunization with a diphtheria, tetanus, acellular pertussis, hepatitis B (DTPa–HBV) and *Haemophilus influenzae* type b (Hib) vaccine administered separately or together is safe and immunogenic

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Immunogenicity

Summary
Objectives: The aim of this study was to evaluate the safety and immunogenicity of DTPa–HBV and Hib vaccines given mixed or separately to 360 healthy infants at 2, 4, and 6 months of age.
Methods: Immune memory was assessed in lower responders (post-primary anti-PRP <0.545 μg/ml), through administration of plain polyribosylribitol phosphate (PRP) at 12–15 months. All subjects received a DTPa–HBV/Hib booster at 18–19 months.
Results: One month after primary vaccination, 98% had seroprotective antibody levels against HBV and 94–97% against Hib (anti-PRP ≥ 0.15 μg/ml). A statistically significant difference between groups was observed in the proportion of subjects who achieved anti-PRP antibodies ≥1.0 μg/ml post-primary vaccination; 68.1% for DTPa–HBV/Hib and 84.5% for DTPa–HBV and Hib. PRP administered to lower responders produced a 7-fold increase in anti-PRP antibodies, indicative of immunological memory. After DTPa–HBV/Hib booster vaccination, 96–100% of subjects had seroprotective antibody concentrations against Hib, hepatitis B, tetanus, and diphtheria and high vaccine response rates against pertussis toxoid, filamentous hemagglutinin, and pertactin.

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Conclusion: A robust and protective Hib response was demonstrated following plain PRP and/or a booster conjugate Hib vaccine in both lower and higher Hib responders.

Introduction

The National Immunisation Program in Australia currently recommends primary vaccination with diphtheria, tetanus and acellular pertussis (DTPa), inactivated polio vaccine (IPV), hepatitis B virus (HBV), *Haemophilus influenzae* type b (Hib), rotavirus and 7-valent pneumococcal conjugate vaccines for infants.[1] A booster dose of Hib is provided at 12 months of age in addition to meningococcal C vaccine and measles, mumps and rubella vaccine. The combination DTPa vaccine was introduced as the standard recommendation in Australia in 1997–1999.[2] A national program for Hib vaccination was begun in 1993, and has resulted in substantial reductions in invasive Hib disease.[3]

The increasing number of vaccines available for immunization against numerous childhood diseases has the effect of making vaccination schedules progressively more complex, which may be a significant barrier to the success of immunization programs.[4] The large number of injections required if each vaccine is administered separately can also be a source of distress to parents and children.[5] The use of combination vaccines can reduce the number of injections, simplify immunization schedules, reduce the risk of delayed doses, improve patient convenience by requiring fewer clinic visits, reduce the perceived pain and distress for the child, and reduce costs associated with vaccine administration.[6] A study of infant immunization in Sydney found that administration of DTP, Hib and HBV vaccines was often fragmented across separate visits, with a risk of missed or delayed doses, and concluded that there was a need for a combination DTPa–HBV–Hib vaccine.[7]

Earlier studies have established the effectiveness of a quadrivalent DTPa–HBV vaccine[8] and the feasibility of combining DTPa–HBV and Hib vaccines in a single injection for primary vaccination in healthy infants.[9–12]

Although the purpose of combination vaccines is to reduce the number of needles administered to children, it is essential that vaccine efficacy is not compromised. It has previously been documented that combining Hib tetanus conjugated vaccines with DTPa vaccines can result in a lower level of circulating antibodies to the capsular polysaccharide polyribosylribitol phosphate (PRP) compared to separate administration of the vaccines. The reasons for the decrease in antibody response in some DTP/Hib combination vaccine studies remain unclear, but the variation in results observed suggests these differences are vaccine-specific.[13] Possible reasons for decreased immunogenicity of Hib in DTPa combination vaccines include direct interference between different antigens when mixed, epitope-specific suppression, and/or variation in adjuvants in vaccines studied.[13] However the variability in response is unlikely to be of any clinical significance as the protective efficacy of DTPa–Hib combination vaccines with lower antibody concentrations has been established.[14,15] In addition, immunological memory has been demonstrated in studies where contact with unconjugated (plain) PRP antigen following priming with a combination Hib vaccine has resulted in induced functional Hib antibody.[16] Plain PRP used in this study and in others as an immunological challenge, is used to mimic exposure to wild-type Hib infection as a method of assessing immunological memory.[17,18]

Data have also shown that when corrected for total antibody level, anti-PRP antibody avidity does not differ with different methods of administration including administration of Hib separately or in combination.[14]

The aim of this study was to investigate the immunogenicity and reactogenicity of a candidate Hib vaccine and quadrivalent DTPa–HBV vaccine given either as a single mixed injection or administered simultaneously in opposite limbs for primary vaccination to healthy infants at 2, 4, and 6 months of age. A follow-up booster study was conducted in which the DTPa–HBV and Hib vaccines were administered as a single injection to the same subjects during the second year of life.

Materials and methods

Study design and subjects

The primary vaccination study (208140/039) was an open-label, randomized, comparative trial conducted in two centers in Australia. Healthy infants of either sex, and aged between 8 and 12 weeks at the time of first immunization, were randomized to receive either a single vaccination with combined DTPa–HBV/Hib or separate injections of DTPa–HBV and Hib in opposite thighs, at 2, 4 and 6 months of age. Subjects were excluded if they had obvious health problems established by clinical examination and/or medical history, or if they had a history of previous exposure to diphtheria, tetanus, pertussis, hepatitis B, or Hib vaccination or disease.

Subjects completing the primary vaccination study were eligible to enter an open-label booster study conducted at the same centers (208108/043) and to receive a single injection of DTPa–HBV/Hib vaccine at 18–19 months of age. To assess immune memory in subjects potentially at risk (i.e., low anti-PRP response after primary vaccination), 40 subjects with anti-PRP values below 0.545 μg/ml after primary vaccination (lower responders) received a dose of plain PRP at 12–15 months of age following the primary vaccination. The cut-off (0.545 μg/ml) was based on the anti-PRP antibody responses of the first 100 subjects evaluated in the primary vaccination study and was chosen so as not to challenge subjects who had already shown a very high response to primary vaccination.

Both study protocols were approved by the ethics committee at each trial center, and the studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The parent or guardian of each subject provided written informed consent before any study procedure was performed.

Study vaccines

All vaccines were manufactured by GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium. The DTPa–HBV licensed vaccine contained, per 0.5 ml dose, diphtheria toxoid ≥30 IU, tetanus toxoid ≥40 IU, pertussis toxoid (PT) 25 μg, filamentous hemagglutinin (FHA) 25 μg, pertactin (PRN) 8 μg, and recombinant surface antigen of the hepatitis B virus (HBsAg) 10 μg. The candidate Hib conjugate vaccine contained, per lyophilized dose, PRP 10 μg conjugated to tetanus toxoid 20–40 μg adsorbed onto aluminum salts as adjuvant, and lactose 10 mg. In addition to the study vaccines all infants received oral polio vaccine in accordance with the Australian Standard Vaccination Schedule.

Immunogenicity assessment

Blood samples for immunogenicity assessment were taken immediately before the first primary vaccine dose and one month after the third dose. In the booster study, blood samples were taken before and one month after the DTPa–HBV/Hib booster vaccine dose. In the subset of subjects receiving plain PRP (10 μg), blood samples were also taken before and 7–10 days after the dose of PRP.

Total antibodies to Hib PRP were measured using a radiolabeled antigen binding assay with a cut-off of 0.15 μg/ml. Antibodies against HBsAg (anti-HBs) were determined using a commercially available radioimmunoassay (AUSAB®, Abbott Laboratories, Abbott Park, IL, USA) with a cut-off of 10 mIU/ml. Antibody concentrations ≥10 mIU/ml were considered as protective. Antibodies against diphtheria and tetanus toxoids were measured by ELISA techniques, with a cut-off level of 0.1 IU/ml. Antibodies (IgG) against PT, FHA, and PRN (pertussis antigens), were measured by ELISA with a cut-off of 5 ELISA units (EL.U)/ml. A vaccine response to PT, PRN, and FHA after the booster dose was defined as appearance of antibodies in initially seronegative subjects or a 2-fold increase in antibody concentration in initially seropositive subjects.

Safety and reactogenicity assessment

Diary cards were distributed to the parents or guardians to record solicited and unsolicited symptoms and adverse events. Reactogenicity was assessed by measuring the appearance of solicited local symptoms (pain, redness, or swelling at the injection site) or general symptoms (irritability/fussiness, fever, vomiting, diarrhea, restlessness, sleepiness, unusual crying, drowsiness, and loss of appetite) during a 4-day follow-up period after each vaccination dose. Unsolicited adverse events (AEs) and serious adverse events (SAEs) were recorded throughout the study period. Intensity was assessed on a three-point scale where grade 3 intensity for solicited symptoms included crying when the limb was moved or a spontaneously painful limb, crying that could not be comforted or that prevented normal everyday activity, drowsiness that prevented normal everyday activity, loss of appetite such that the study subject did not eat at all. Local redness and swelling were assessed by measuring the largest diameter, where grade 3 was >20 mm.

Statistical analyses

The study was exploratory. Antibody seroprotection/seropositivity and vaccine response rates against vaccine antigens for the according-to-protocol (ATP) cohort were calculated with exact 95% confidence intervals (CI). Geometric mean antibody concentrations (GMC) with 95% CI were calculated from the anti-log of the mean of log-transformed values. Antibody concentrations below the lower limit of detection of the assay were assigned an arbitrary value of half the cut-off for the purpose of GMC calculation.

Reactogenicity was evaluated by calculating the percentage (and 95% CI) of doses followed by a report of at least one solicited or unsolicited local or general symptom during the defined follow-up period after vaccination. For each group, the incidence of each symptom overall and rated as grade 3 was recorded. Values were considered as significantly different between groups if the 95% CI did not overlap.

Results

Demographics

The primary study was conducted between November 1996 and January 1998, and enrolled 360 infants. The booster study took place between January 1998 and January 1999 and included 276 subjects. Figure 1 presents the disposition of subjects in both studies. All but three infants completed the primary vaccination course (all were lost to follow-up). The ATP cohort for immunogenicity comprised 328 (91.1%) infants. Thirty-two subjects were excluded from the ATP analysis (randomization failure (1), initially seropositive or entry status unknown (4), prohibited medication (1), non-compliant with vaccination or blood sampling schedule (21), and essential serological data missing (5)).

In the primary study, the mean age at first dose was 8.6 weeks and 168/328 (51.2%) were male. The mean age at the time of PRP challenge was 13.4 months in the subgroup that received plain PRP vaccination, and the mean age at the time of booster vaccination was 17.9 months and 18.0 months in the subjects primed with DTPa–HBV + Hib and DTPa–HBV/Hib, respectively.

Immunogenicity

Primary vaccination

Anti-PRP and anti-HBs antibody concentrations were evaluated prior to and after the primary vaccination course. Seroprotection rates and GMCs one month after the third dose of primary vaccination are presented in Table 1.

A total of 94.4% (95% CI 89.6–97.4%) of subjects in the combined DTPa–HBV/Hib group had seroprotective antibody concentrations against Hib (anti-PRP ≥0.15 μg/ml) compared with 97.6% (95% CI 94.0–99.3%) in the group receiving separate injections. A higher proportion of subjects in the group that received separate injections reached an antibody level against PRP of ≥1.0 μg/ml (84.5%, 95% CI 78.2–89.6%) compared with the group that received the combined injection (68.1%, 95% CI 60.3–75.3%). The GMC for anti-PRP antibodies was also higher in the group that received separate injections (4.553 μg/ml (95% CI 3.647–5.685 μg/ml))

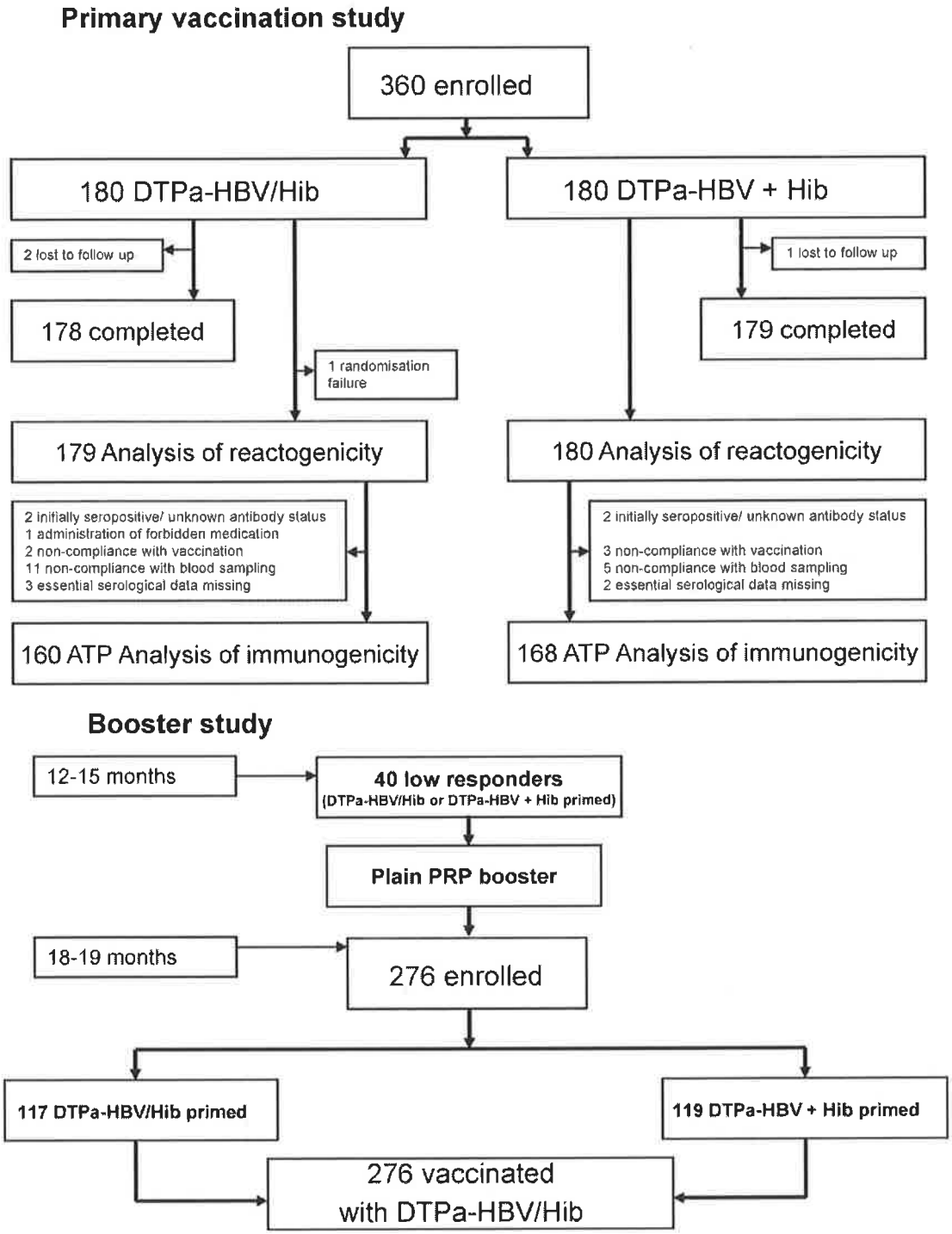

Figure 1 Disposition of subjects included in the primary and booster vaccination trials.

than in the group that received the combined injection (2.034 μg/ml (95% CI 1.574–2.628 μg/ml)).

Anti-HBs antibody levels of ≥10 mIU/ml one month after the third dose were high in both groups, occurring in 98.8% of subjects receiving a single injection and 98.2% of subjects receiving separate injections.

Plain PRP challenge: assessment of immune memory

Infants younger than 18–24 months of age cannot mount a seroprotective response to plain polysaccharides such as PRP unless immune memory has been previously primed. The presence of immune memory was assessed in the subset of 40 subjects that were lower responders, by measuring the increase in anti-PRP antibody concentrations 7 days after the administration of plain PRP at 12–15 months of age. Twenty-five lower responders had received priming with combined DTPa–HBV/Hib vaccine and 15 had received DTPa–HBV + Hib vaccines. The results show that after a PRP challenge, the anti-PRP antibody GMC rapidly increased, indicating that anti-PRP immune memory had been induced regardless of

Table 1 Seroprotection/seropositivity rates and antibody geometric mean concentrations one month after the third dose of DTPa–HBV and Hib vaccines (ATP cohort for immunogenicity)

	DTPa–HBV/Hib (N = 160)				DTPa–HBV + Hib (N = 168)			
Antigen	%	95% CI	GMC	95% CI	%	95% CI	GMC	95% CI
Anti-PRP								
≥0.15 μg/ml	94.4	89.6–97.4	2.034	1.574–2.628	97.6	94.0–99.3	4.553	3.647–5.685
≥1.0 μg/ml	68.1[a]	60.3–75.3	-	-	84.5[a]	78.2–89.6	-	-
Anti-HBs								
≥10 mIU/ml	98.8	95.6–99.8	920.3	752.1–1126.1	98.2	94.9–99.6	783.6	629.7–975.0

DTPa–HBV, diphtheria, tetanus, acellular pertussis, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine; ATP, according-to-protocol; *N*, number of subjects with available results; 95% CI, 95% confidence interval; GMC, geometric mean concentration; anti-PRP, antibodies to polyribosylribitol phosphate; anti-HBs, antibodies to the hepatitis B surface antigen.
[a] Statistically significant difference – 95% CI do not overlap.

Table 2 Anti-PRP seroprotection rates and antibody geometric mean concentrations after the plain PRP challenge in subjects classified as lower responders (anti-PRP <0.545 μg/ml) one month after the primary vaccination course

Post-primary antibody concentration		Anti-PRP ≥0.15 μg/ml		GMC (μg/ml)	
	n	%	95% CI	GMC	95% CI
<0.15 μg/ml	7	85.7	42.1–99.6	0.372	0.155–0.893
≥0.15 to <0.545 μg/ml	32	96.9	83.8–99.9	1.296	0.729–2.305
Total	39	94.9	82.7–99.4	1.036	0.624–1.722

PRP, polyribosylribitol phosphate; GMC, geometric mean concentration; 95% CI, 95% confidence interval.

whether the subjects were primed with DTPa–HBV/Hib or DTPa–HBV + Hib (Table 2; Figure 2). Before the plain PRP challenge, 50% (95% CI 33.4–66.6%) of the lower responders had anti-PRP ≥0.15 μg/ml, which rose to 94.9% (82.7–99.4%) 7 days post-PRP challenge (Table 2). Of low responders with post-primary anti-PRP antibodies <0.15 μg/ml, 85.7% achieved seroprotective concentrations after the challenge dose. In low responders the anti-PRP antibody GMC increased 7-fold from 0.146 μg/ml (95% CI 0.113–0.187 μg/ml) pre-vaccination to 1.036 μg/ml (95% CI 0.624–1.722 μg/ml) post-vaccination.

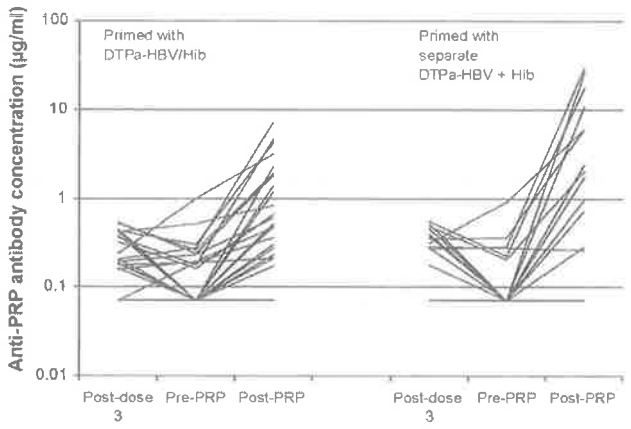

Figure 2 Individual responses to plain PRP challenge by vaccine used for primary vaccination. Post-dose 3 = one month after primary vaccination; Pre-PRP and post-PRP = prior to and 7 days after challenge with plain PRP at 12–15 months of age. Each line represents results from an individual.

Booster vaccination

Prior to booster dosing with DTPa–HBV/Hib, antibody persistence was similar in both groups, irrespective of whether the primary vaccination had been administered using separate or single injections (Table 3). One month after booster vaccination, substantial increases in all antibody concentrations were observed. The percentage of subjects with anti-PRP ≥1.0 μg/ml reached 98.2–100% after boosting. At least 99.1% of subjects had seroprotective antibody concentrations against diphtheria, tetanus and hepatitis B after the booster dose. Vaccine response rates as represented by anti-PT, anti-FHA and anti-PRN antibody concentrations were also high (over 96%). Overall, the immune response to the booster dose was similar for the two comparative treatment groups.

Safety and reactogenicity

Primary vaccination

Primary vaccination was well tolerated in both groups (Table 4). The incidence of grade 3 solicited symptoms was low, and was similar in both groups (Table 4). There were no statistically significant differences in reactogenicity between the two groups.

The total number of unsolicited symptoms reported in the 30-day follow-up period after primary vaccination was 253 in the group receiving the vaccines as a single injection and 228 in the group receiving separate injections.

A total of 27 SAEs were reported during the study. All except one were considered unrelated to vaccination by the investigator. One SAE was assessed as probably related, a hypotonic hyporesponsive episode that occurred in the separate vaccination group. The event lasted for 45 minutes,

Table 3 Seroprotection/seropositivity and antibody geometric mean concentrations before and one month after booster vaccination with DTPa–HBV/Hib vaccine in children aged 18–19 months (total cohort)

		DTPa–HBV/Hib primed					DTPa–HBV + Hib primed				
	Time point	*N*	%	95% CI	GMC	95% CI	*N*	%	95% CI	GMC	95% CI
Anti-PRP	Pre-booster	103	81.6	72.7–88.5	0.464	0.365–0.591	113	86.7	79.1–92.4	0.481	0.383–0.604
≥0.15 μg/ml	Post-booster	114	100.0	96.8–100.0	70.286	55.522–88.977	111	100.0	96.7–100.0	78.544	64.275–95.981
Anti-PRP	Pre-booster	103	24.3	16.4–33.7	-	-	113	23.0	15.6–31.9	-	-
≥1.0 μg/ml	Post-booster	114	98.2	93.8–99.8	-	-	111	100.0	96.7–100.0	-	-
Anti-T	Pre-booster	105	74.3	64.8–82.3	0.158	0.134–0.187	112	88.4	81.0–93.7	0.235	0.201–0.274
≥0.1 IU/ml	Post-booster	114	100.0	96.8–100	5.762	4.956–6.699	111	99.1	95.1–100	8.559	7.364–9.948
Anti-D	Pre-booster	104	43.3	33.6–53.3	0.089	0.078–0.103	112	37.5	28.5–47.1	0.082	0.072–0.094
≥0.1 IU/ml	Post-booster	114	100.0	96.8–100	4.369	3.712–5.143	111	100.0	96.7–100	3.422	2.912–4.022
Anti-HBs	Pre-booster	105	85.7	77.5–91.8	76.7	55.4–106.2	113	88.5	81.1–93.7	70.8	53.8–93.4
≥10 mIU/ml	Post-booster	114	100.0	96.8–100	2365.4	1696.7–3297.8	112	100.0	96.8–100	1568.4	1155.3–2129.3
Anti-PT	Pre-booster	104	48.1	38.2–58.1	5.2	4.3–6.2	113	46.9	37.5–56.5	4.8	4.1–5.5
≥5 EL.U/ml	Post-booster	114	100.0	96.8–100.0	70.3	60.6–81.6	111	99.1	95.1–100.0	61.9	53.0–72.3
VR	Post-booster	104	96.2	90.4–98.9	-	-	106	99.1	94.9–100.0	-	-
Anti-FHA	Pre-booster	101	99.0	94.6–100.0	40.2	33.5–48.1	108	100.0	96.6–100.0	41.1	35.3–47.8
≥5 EL.U/ml	Post-booster	114	100.0	96.8–100.0	822.0	724.6–932.4	111	100.0	96.7–100.0	708.8	614.0–818.2
VR	Post-booster	101	97.0	91.6–99.4			101	98.0	93.0–99.8		
Anti-PRN	Pre-booster	105	91.4	84.4–96.0	16.7	14.1–19.8	113	89.4	82.2–94.4	16.1	13.4–19.3
≥5 EL.U/ml	Post-booster	114	100.0	96.8–100.0	776.4	659.6–913.8	111	100.0	96.7–100.0	632.7	530.3–754.8
VR	Post- booster	105	99.0	94.8–100.0	-	-	106	98.1	93.4–99.8	-	-

DTPa–HBV, diphtheria, tetanus, acellular pertussis, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine; *N*, number of subjects with available serology results at the specified time point; %, percentage of subjects with specified antibody concentrations or vaccine response; 95% CI, 95% confidence interval; GMC, geometric mean concentration; anti-PRP, antibodies to polyribosylribitol phosphate; anti-T, antibodies to tetanus toxoid; anti-D, antibodies to diphtheria toxoid; anti-HBs, antibodies to the hepatitis B surface antigen; anti-PT, antibodies to the pertussis toxoid; anti-FHA, antibodies to filamentous hemagglutinin; anti-PRN, antibodies to pertactin; VR, vaccine response (appearance of antibodies in initially seronegative subjects or a 2-fold increase in antibody concentration in initially seropositive subjects); EL.U, ELISA units; IU, international units.

Table 4 Incidence of solicited local and general symptoms within the 4-day follow-up: primary vaccination (overall doses, ATP reactogenicity cohort) and booster vaccination (total cohort)

	Primary vaccination						Booster vaccination with DTPa–HBV/Hib			
	DTPa–HBV/Hib (*N* = 533)		DTPa–HBV + Hib (*N* = 538)				DTPa–HBV/ Hib-primed (*N* = 116)		DTPa–HBV + Hib-primed (*N* = 117)	
			DTPa–HBV		Hib					
Symptom	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Pain	19.5	16.2–23.1	16.5	13.5–20.9	12.6	10.0–15.7	50.0	40.6–59.4	53.8	44.4–63.1
Grade 3[a]	0.2	0.0–1.0	1.1	0.4–2.4	0.4	0.0–1.3	6.0	2.5–12.0	4.3	1.4–9.7
Redness	38.6	34.5–42.9	35.1	31.1–39.3	25.1	21.5–29.0	70.7	61.5–78.8	69.2	60.0–77.4
>20 mm	2.3	1.2–3.9	1.7	0.8–3.2	0.9	0.3–2.2	29.3	21.2–38.5	26.5	18.8–35.5
Swelling	25.3	21.7–29.2	23.4	19.9–27.2	11.0	8.5–13.9	54.3	44.8–63.6	56.4	46.9–65.6
>20 mm	4.5	2.9–6.6	3.7	2.3–5.7	0.4	0.0–1.3	25.9	18.2–34.8	25.6	18.0–34.5
Diarrhea	17.1	14.0–20.5	16.2	13.2–19.6	8.6	4.2–15.3	9.3	4.7–16.1		
Grade 3[b]	0.4	0.0–1.3	0.4	0.0–1.3	0.0	0.0–3.1	0.0	0.0–3.1		
Fever[c]										
≥37.5 °C	14.4	11.6–17.7	11.2	8.6–14.1	18.1	11.6–26.3	16.9	10.7–25.0		
>39.0 °C	0.4	0.0–1.3	0.7	0.2–1.9	0.9	0.0–4.7	2.5	0.5–7.3		
Fussiness	52.0	47.6–56.3	54.6	50.3–58.9	51.7	42.3–61.1	44.1	34.9–53.5		
Grade 3[b]	0.9	0.3–2.2	2.0	1.3–3.6	2.6	0.5–7.4	0.0	0.0–3.1		
Loss of appetite	14.1	11.2–17.3	15.1	12.1–18.4	31.0	22.8–40.3	22.0	14.6–30.6		
Grade 3[b]	0.4	0.0–1.3	0	0.0–0.7	1.7	0.2–6.1	1.7	0.2–6.0		
Restlessness	31.5	27.6–35.7	31.8	27.9–35.9	26.7	18.9–35.7	31.4	23.1–40.5		
Grade 3[b]	0.4	0.0–1.3	0	0.0–0.7	4.3	1.4–9.8	0.8	0.0–4.6		
Sleeping more than usual	28.1	24.4–32.2	28.3	24.5–32.3	19.8	13.0–28.3	16.1	10.0–24.0		
Grade 3[b]	0.2	0.0–1.0	0.2	0.0–1.0	0.9	0.0–4.7	0.8	0.0–4.6		
Unusual crying	37.0	32.9–41.2	40.7	36.5–45.0	10.3	5.5–17.4	7.6	3.5–14.0		
Grade 3[b]	0.4	0.0–1.3	0.6	0.1–1.6	2.6	0.5–7.4	0.0	0.0–3.1		
Vomiting	14.1	11.2–17.3	13.9	11.1–17.2	7.8	3.6–14.2	4.2	1.4–9.6		
Grade 3[b]	0.4	0.0–1.3	0	0.0–0.7	0.9	0.0–4.7	0.0	0.0–3.1		

ATP, according-to-protocol; DTPa–HBV, diphtheria, tetanus, acellular pertussis, hepatitis B vaccine; Hib, *Haemophilus influenzae* type b vaccine; *N* = total number of diary cards returned following all doses (results presented from subjects who did not receive PRP challenge); 95% CI, 95% confidence interval; PRP, polyribosylribitol phosphate.

[a] Grade 3 pain at injection site = pain such that the infant cries when the limb is moved.

[b] Grade 3 = symptom that prevents normal everyday activities.

[c] Fever = axillary temperature.

after which the subject recovered. No subject withdrew from the study due to an AE.

Booster vaccination

Local symptoms were reported more commonly after the booster dose, with pain redness or swelling reported by up to 70.7% of subjects (Table 4). In contrast, with the exception of loss of appetite and fever, the incidence of general solicited symptoms tended to be lower than that following primary vaccination. Fever >39.0 °C and grade 3 loss of appetite were uncommon. There was no appreciable difference between groups (primed with DTPa–HBV/Hib or DTPa–HBV + Hib) in terms of the incidence or intensity of solicited symptoms that occurred after the booster dose of DTPa–HBV/Hib. Unsolicited clinical events were reported by 102 subjects in the DTPa–HBV/Hib-primed group and 119 subjects in the DTPa–HBV + Hib-primed group. One event (otitis media) was of grade 3 intensity, but was not considered by the investigator to be related to the booster dose. A total of nine SAEs were reported, none of which were considered related to the study vaccine. All subjects recovered, and none withdrew from the study due to a SAE.

Discussion

A large range of combination vaccines, including DTPa–HBV, DTPa–IPV, and DTPa–HBV–IPV (GlaxoSmithKline Biologicals) have been developed, any of which can be reconstituted with lyophilized conjugate Hib vaccine to provide protection

against up to six diseases in a single injection.[10] The efficacy and safety of such DTPa-based combinations have been established in a range of clinical studies in infants, toddlers, and school-age children.[11,19–22]

The study reported here evaluated the immunogenicity and safety of DTPa–HBV and Hib vaccines administered as a single mixed injection or as separate injections in opposite limbs for primary vaccination in infants aged 2, 4, and 6 months. A follow-up study investigated the immunogenicity and safety of booster vaccination with DTPa–HBV/Hib as a single injection given in the second year of life. This study is original in that plain PRP was used to demonstrate an anamnestic response to a primary course of Hib vaccine in children identified as non-responders and low responders to Hib, prior to a booster Hib vaccination.

Primary immunization with the combined vaccine resulted in high levels of seroprotection (94%) against the Hib PRP antigen (defined as anti-PRP antibody $\geq$0.15 μg/ml) and 97% seroprotection for separate injections. Similarly for hepatitis B, no statistically significant difference was observed between the combined or separate methods of vaccine administration with overlapping 95% CI for seroprotection rate. However the proportion of subjects with anti-PRP antibody levels of $\geq$1.0 μg/ml, and anti-PRP antibody GMC, was higher in the group that received separate injections. Concentrations of specific anti-PRP antibody >0.15 μg/ml have traditionally been associated with short-term protection against natural infections, whereas concentrations >1.0 μg/ml have been associated with long-term protection.[23,24] It is well documented that the combined administration of Hib tetanus-conjugated vaccines with DTPa-based vaccines can reduce the level of circulating antibodies to PRP compared to separate administration of the Hib vaccine.[11,14,25] However, it has also been shown that the functional nature of the antibodies against Hib produced by combined DTPa-based/Hib vaccines is the same as those induced by separate injections, and that immunological memory is induced.[15,16]

The results of the present study confirm these findings and in addition demonstrate induction of immune memory to the PRP antigen even in the lower responders. Indeed, a subset of subjects with lower anti-PRP responses to primary vaccination (defined using an arbitrary cut-off to identify the 10% of lowest responders in the primary vaccination study) who received an injection of plain PRP at age 12–15 months showed an increase in mean anti-PRP GMC of over 7-fold. This response to PRP challenge, at an age where no significant response to the polysaccharide is expected, is indicative of immunological memory and confirms the findings of other studies.[22] The dose of plain PRP in this study, mimicking Hib infection, was used to demonstrate effective priming and immunological memory in subjects who developed a lower antibody response to Hib. The anamnestic response observed following plain PRP challenge and the booster DTPa–HBV/Hib dose confirm results from other studies with DTPa–Hib combination vaccines showing induction of immune memory.[17,25,26] In addition studies that have examined the functional and qualitative characteristics of antibodies have shown no difference between separate or mixed Hib vaccine administration.[15]

The importance of a booster dose of Hib conjugate vaccine in achieving effective and long-term immunity is well appreciated. Missing the recommended booster dose was associated with an increase in Hib disease in Germany[27] and with a reduction in prevention of Hib colonization.[28] Immunity after primary vaccination without booster was shown to wane over time in the UK, with a fall in vaccine effectiveness to 37.3% two years post-vaccination.[28,29] Concerns about the efficacy of Hib in DTPa-based/Hib combination vaccines have been negated by epidemiological data showing that when these vaccines have been included in routine infant schedules they have been highly successful in prevention of Hib disease.[30]

In terms of other vaccine antigens, the proportion of subjects who developed seroprotective antibody concentrations against diphtheria, tetanus, and hepatitis B or a vaccine response to pertussis antigens after the booster dose was high, and robust increases in antibody concentrations were observed regardless of the vaccine administered for the primary vaccination course.

Mixing of the DTPa–HBV and Hib vaccines did not result in increased reactogenicity for either primary or booster vaccination. In the booster study reported here, the combined DTPa–HBV/Hib booster vaccination was found to be safe, with no SAEs that were considered to be related to treatment, and no withdrawals due to SAEs. There was however, a notable increase in grade 3 pain and redness and swelling >20 mm following the booster vaccinations in both groups. Booster DTPa vaccination has been shown to be associated with a higher rate of extensive local reactions than primary vaccination, the pathogenesis of which is likely to be multifactorial.[31,32] However, these local reactions have been shown to resolve spontaneously without any resulting sequelae.[33]

In conclusion, DTPa–HBV and Hib vaccines have been shown to be safe and immunogenic whether administered as a single injection or as separate injections for primary vaccination of infants at ages 2, 4, and 6 months. After booster vaccination with combined DTPa–HBV/Hib vaccine at age 18–19 months, 96–100% of subjects showed seroprotective/seropositive levels of antibodies against all vaccine antigens, and induction of immune memory to PRP was demonstrated in low–moderate responders.

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A comparison of booster immunisation with a combination DTPa-IPV vaccine or DTPa plus IPV in separate injections when co-administered with MMR, at age 4–6 years

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Abstract

This study evaluated GSK's combined DTPa-IPV vaccine (Infanrix™-IPV) given as a fifth consecutive acellular pertussis booster dose in conjunction with the second dose of MMR vaccine (Priorix™) in children aged 4–6 years. The immunogenicity and reactogenicity of this vaccine regimen was compared with separate injections of DTPa and IPV when given concomitantly with MMR. A cohort of 362 children previously primed with four doses of DTPa and OPV, and a single dose of MMR were randomized to receive either DTPa-IPV + MMR ($N = 181$) or DTPa + IPV + MMR ($N = 181$). Antibody concentrations were measured prior to and 1 month after the booster dose. After immunisation all subjects from both groups had seroprotective antibody levels against diphtheria, tetanus and the three poliovirus serotypes, ≥96% showed vaccine response to PT, FHA and PRN, all were seropositive to mumps and rubella, and all but one subject were seropositive to measles. Immunogenicity results for each component antigen were similar for DTPa-IPV and separately co-administered DTPa and IPV. Local reactions were common with 24.0% and 31.1% of children experiencing swelling >50 mm at the DTPa-IPV and DTPa injection sites, respectively. The DTPa-IPV combination did not increase the incidence or intensity of adverse events compared with separately administered DTPa + IPV. The response to the concomitantly administered MMR vaccine was similar in the two groups and similar to previously reported responses for a second dose of MMR. This combined DTPa-IPV vaccine has a similar reactogenicity profile to DTPa, is immunogenic when given as a booster dose at 4–6 years of age, and has no impact on the immunogenicity of a co-administered second dose of MMR vaccine.

Keywords: DTPa-IPV; Booster; Measles-mumps-rubella; Local swelling reactions

1. Introduction

A number of immunisation schedules now include a recommendation for inactivated polio vaccine to be administered to infants as "best practice" [1]. Inactivated polio vaccine (IPV) is increasingly preferred over oral polio vaccine (OPV) due to the very rare possibility of vaccine associated paralytic poliomyelitis (VAPP) in recipients of OPV and their contacts [1,2]. In the USA, where 8–10 cases of VAPP were reported each year, IPV has been the recommended polio vaccine since 1997 [2]. In Europe, a number of countries have moved from using OPV to using IPV in the last decade, with IPV alone being now used in Austria, Belgium, Finland, France, Germany, Iceland, Luxembourg, Monaco and The Netherlands [3]. In the United Kingdom

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(UK), IPV was recently included in the routine immunisation schedule due to the relative increase in importance of VAPP as the number of cases of poliomyelitis due to wild virus decreased [4].

According to data from the Australian Childhood Immunisation Register (ACIR) [5], 99.2% of children in Australia, still receive OPV despite a recommendation for IPV (without provision of funding) since September 2003 [5]. IPV will be funded Federally for all Australian children from November 2005, but the successful implementation of this recommendation would be facilitated by the availability of suitable combination vaccines to avoid an increase in the number of injections provided at routine immunisation visits and a more cost-effective alternative to monovalent poliovirus vaccine. Combination vaccines, by reducing the number of injections given at each immunisation encounter, increase convenience of immunisations for both the vaccinees and immunisation providers [6,7]. In doing so, they have the advantage of potential higher compliance with immunisation programs and reduction of their overall costs [2,8,9]. Administration of fewer vaccines simplifies storage and delivery logistics, fewer staff are required for immunisation delivery and the risk of immunisation related errors is reduced. There is evidence that some parents and immunisation providers are reluctant for children to have multiple injections at an immunisation encounter [6,7]. This may result in delayed completion of immunisations with increased risk to the infant of acquiring a vaccine preventable disease [10]. Several IPV-containing combination vaccines are available, including a combined DTPa-IPV vaccine for primary and booster immunisation, but the impact of new combination vaccines on the immunogenicity and reactogenicity of other co-administered vaccines needs to be evaluated.

Provision of a pertussis containing vaccine prior to school entry is required to provide protection against pertussis infection and improve herd immunity in the population [11]. Previous studies have shown that large local reactions may occur with the administration of the fourth or fifth dose of a variety of DTPa vaccines. These reactions are more likely to occur when DTPa is provided for all primary and booster doses compared to a combination of DTPa and DTPw [12–19]. Despite the increased incidence of large local reactions, swelling and discomfort usually resolve without sequelae. Rarely, swelling may involve the whole limb and may be associated with pain and limitation of movement.

In this study, the immunogenicity and reactogenicity of DTPa-IPV vaccine when administered as a booster at 4–6 years of age was examined and compared to DTPa and IPV administered separately. These vaccines were co-administered with measles, mumps, rubella (MMR) vaccine given as a second dose, prior to school entry. The aims of the study were to compare the safety and immunogenicity of the study vaccines administered in both groups and to assess the reactogenicity and immunogenicity of MMR vaccine 1 month after immunisation.

2. Methods

The study was an open, randomized phase IIIb, non-inferiority study conducted in three centres in Australia. Children free of obvious health problems and who had completed a primary vaccination course with DTPa and polio vaccines at 2, 4 and 6 months of age and had received a DTPa booster and immunisation for MMR in the second year of life were included in the study.

The study was approved by the individual hospital ethics committees at the three study centres, and was conducted in accordance with Good Clinical Practice guidelines. Written informed consent was obtained from a parent/guardian of each subject. The DTPa-IPV and DTPa vaccines (GlaxoSmithKline Biologicals, GSK) contained ≥30 IU of diphtheria toxoid, ≥40 IU of tetanus toxoid, 25 μg pertussis toxoid (PT), 25 μg filamentous haemagglutinin (FHA) and 8 μg of pertactin (PRN). The DTPa-IPV and the IPV vaccine (manufactured by Aventis Pasteur MSD), each contained 40 D Ag units of poliovirus type 1; 8 D Ag units of poliovirus type 2; 32 D Ag units of poliovirus type 3. The MMR vaccine (GSK) contained ≥103.0 TCID50 of Schwarz measles strain, ≥103.7 TCID50 of RIT 4385 mumps strain and ≥103.0 TCID50 of RA 27/3 rubella strain.

The DTPa-IPV and DTPa vaccines were administered as deep intramuscular injections into the left deltoid region using a 23 gauge, 25 mm length needle. The IPV and MMR vaccines were administered as subcutaneous injections in the lower right deltoid and the upper right deltoid region, respectively, at least 25 mm apart, using 25 gauge, 16 mm length needles.

Randomisation was performed centrally using an algorithm with a minimisation procedure stratified by centre [20].

Serum samples were obtained prior to and between 21 and 48 days following immunisation. In house assays for antibodies to diphtheria, tetanus, PT, FHA and PRN were performed in the laboratories of Dr. M. Pichichero (University of Rochester, New York) and at GSK Biologicals laboratories (Rixensart, Belgium) for antibodies to poliovirus types 1–3, using validated methods described elsewhere [21]. Assays for measles, mumps and rubella (Enzygnost™) were performed at GSK Biologicals laboratories.

Seroprotection status was defined for poliovirus types 1–3 as an antibody titre ≥8; and for diphtheria and tetanus toxoids as an antibody concentration ≥0.1 IU/ml.

Seropositivity status was defined for PT, FHA and PRN as an antibody concentration ≥5 EL U/ml; for mumps as an antibody concentration ≥231 U/ml, for measles as an antibody concentration ≥150 mIU/ml and for rubella as an antibody concentration ≥4 and ≥10 IU/ml. The secondary endpoints for the study also included anti-poliovirus types 1–3 antibody concentrations and anti-diphtheria toxoid, anti-tetanus toxoid, anti-measles, anti-mumps and anti-rubella antibody concentrations. A vaccine response to PT, PRN and FHA was defined as appearance of antibodies in initially seronegative

subjects or a two-fold increase in antibody concentration in initially seropositive subjects.

Diary cards were distributed to the parents or guardians to record solicited and unsolicited symptoms and adverse events. Safety was evaluated by measurement of the appearance of solicited local (pain, redness and swelling at the injection site) and systemic adverse events (irritability/fussiness, drowsiness and loss of appetite) on the day of vaccination and for 3 subsequent days. Local redness and swelling were assessed by measurement of the largest diameter of the injection site reaction.

Occurrences of specific solicited general symptoms were also elicited. Parents or guardians observing large swelling reactions (defined as swelling with a diameter of >50 mm, noticeable diffuse swelling or noticeable increase in limb circumference) were asked to contact study staff to allow assessment of the swelling reaction. Fever (axillary body temperature) and MMR specific symptoms: rash/exanthema, parotid/salivary gland swelling and signs of meningism were also solicited and recorded on the day of vaccination and the 14 subsequent days.

Unsolicited adverse events and serious adverse events were recorded for 30 days following vaccination.

2.1. Statistical methods

Statistical analysis was performed using SAS System software [22]. Analysis was carried out on the vaccinated cohort and According To Protocol (ATP) cohorts. The primary cohort for immunogenicity was the ATP immunogenicity cohort. The ATP safety cohort was primarily used for safety analysis. The geometric mean antibody concentration/titres (GMC/GMTs) for each vaccine antigen and virus were calculated with the respective 95% confidence intervals (CI). Seropositivity/seroprotection rates for antibodies against each vaccine antigen, and vaccine response rates to PT, FHA and PRN, 1 month after vaccination were calculated for each group with the corresponding 95% CIs.

Immune responses following the combined DTPa-IPV vaccine were compared to those induced by the separate administration of DTPa and IPV based on standardised asymptotic two-sided 95% CIs calculated for the differences in post-vaccination seroprotection rates between groups and on 95% CIs computed for the ratio of post-vaccination antibody GMCs adjusted for the pre-vaccination antibody concentrations, using a one-way ANOVA model on the logarithm 10 transformation of the antibody concentrations. The model included the group as fixed effect and the pre-vaccination antibody concentration as regressor.

Non-inferiority was defined as occurring if 1 month after vaccination the upper limit of two-sided 95% CI for the absolute group difference in seroprotection rates was below 10% for each of the diphtheria, tetanus and polio antigens and the upper limit of two-sided 95% CI for the group GMC ratio was below 1.5 for each of the pertussis antigens. With a sample size of 366 subjects, this study had >80% overall power to meet the primary objective and demonstrate non-inferiority of the DTPa-IPV vaccine compared to the separate administration of DTPa and IPV.

All safety/reactogenicity analyses were descriptive, with incidences of solicited/unsolicited symptoms and corresponding 95% CIs calculated per group.

3. Results

3.1. Study population

Three urban centres in Australia (Adelaide, Melbourne and Perth) enrolled a total of 366 healthy children 4–6 years of age (56% female) between May and December 2002 and 362 subjects received a dose of the study vaccine (four subjects were eliminated prior to receiving a vaccination as they did not fulfil the inclusion/exclusion criteria). Of the 362 subjects vaccinated, 360 subjects completed the study. One in each group withdrew from the study for reasons not related to adverse events. The mean age of the ATP safety and immunogenicity cohorts at the time of vaccination was 4.2 years (standard deviation 4.2 months, range 4–6 years).

Almost 98% of subjects in each group were Caucasian.

Of the 362 subjects vaccinated, 338 subjects (171 in the DTPa-IPV group and 167 in the DTPa + IPV group) were included in the ATP safety cohort. Twenty-four subjects were eliminated from the ATP safety analysis for the following reasons; having previously received a DTPw vaccine or a DTP vaccine of unknown nature, having already received a fifth dose of DTPa, or had not returned diary cards (subjects who withdrew from the study). Of the 338 subjects included in the ATP safety cohort, 329 subjects (166 in the DTPa-IPV group and 163 in the DTPa + IPV group) were included in the ATP immunogenicity analysis. Nine subjects were eliminated as they were out of the interval for the post-vaccination blood sampling ($n = 4$) or because no serology data were available ($n = 5$).

There were no statistically significant differences in the demographic characteristics of the vaccinated cohort and ATP cohort (data not shown).

3.2. Imunogenicity

Tables 1–3 show the immunogenicity results (seropositivity/seroprotection rates and GMC/GMTs) for antibodies against diphtheria, tetanus, PT, FHA, PRN, polio types 1–3, measles, mumps and rubella. Despite the initial high seroprotection and seropositivity rates, marked increases in post-vaccination GMC/GMTs were observed for all vaccine antigens (Tables 1 and 2). Prior to booster immunisation, seroprotection and seropositivity rates were similarly high in both the DTPa-IPV and DTPa + IPV groups, seroprotective rates ranging from 64.4% to 68.2% for diphtheria and 85.5–100% for all other antigens (Table 1). One month following immunisation, all subjects in both groups had seroprotective antibody

Table 1
Pre- and post-booster seroprotection/seropositivity rates and antibody GMCs prior to and 1 month after booster vaccination (ATP immunogenicity cohort)

Antibody	Timing	Group DTPa-IPV + MMR					Group DTPa + IPV + MMR				
		Seroprotected/seropositive			GMC/T		Seroprotected/seropositive			GMC/T	
		N	%	95% CI	Value	95% CI	*N*	%	95% CI	Value	95% CI
Anti-diphtheria	Pre-booster	160	64.4	56.4, 71.8	0.189	0.155, 0.230	154	68.2	60.2, 75.4	0.211	0.171, 0.260
≥0.1 IU/ml	Post-booster	165	100	97.8, 100	5.931	5.092, 6.908	159	100	97.7, 100	5.998	5.130, 7.013
Anti-tetanus	Pre-booster	160	90.6	85.0, 94.7	0.355	0.303, 0.415	154	93.5	88.4, 96.8	0.411	0.349, 0.483
≥0.1 IU/ml	Post-booster	165	100	97.8, 100	7.884	7.120, 8.730	159	100	97.7, 100	6.750	6.150, 7.410
Anti-PT	Pre-booster	160	51.3	43.2, 59.2	5.4	4.7, 6.3	152	51.5	43.1, 59.5	5.4	4.7, 6.3
≥5 EL U/ml	Post-booster	165	99.4	96.7, 100	110.9	94.7, 129.8	159	100	97.7, 100	127.9	112.4, 145.5
Anti-FHA	Pre-booster	160	97.5	93.7, 99.3	35.3	30.0, 41.7	153	95.4	90.8, 98.1	28.6	24.4, 33.5
≥5 EL U/ml	Post-booster	165	100	97.8, 100	372.0	330.8, 418.3	158	100	97.7, 100	409.6	362.9, 462.3
Anti-PRN	Pre-booster	160	100	97.7, 100	39.6	33.9, 46.2	154	96.8	92.6, 98.9	38.2	32.4, 45.2
≥5 EL U/ml	Post-booster	165	100	97.8, 100	707.0	619.2, 807.4	159	100	97.7, 100	663.0	575.8, 763.5
Anti-poliovirus	Pre-booster	155	87.1	80.8, 91.9	53.9	42.0, 69.2	152	85.5	78.9, 90.7	45.6	35.3, 58.8
Type 1 ≥8	Post-booster	156	100	97.7, 100	3014.4	2591, 3507	151	100	97.6, 100	3218.8	2757.8, 3756.8
Anti-poliovirus	Pre-booster	155	92.3	86.9, 95.9	82.6	64.5, 105.7	154	93.5	88.4, 96.8	91.1	70.4, 117.8
type 2 ≥8	Post-booster	153	100	97.6, 100	2883.2	2484.2, 3346.1	152	100	97.6, 100	3531.7	3075.3, 4055.9
Anti-poliovirus	Pre-booster	155	89.7	83.8, 94.0	42.9	34.5, 53.5	152	90.8	85.0, 94.9	47.2	37.4, 59.5
Type 3 ≥8	Post-booster	148	100	97.5, 100	4848.7	4258.4, 5520.9	147	100	97.5, 100	4865.2	4295.8, 5510.1
Anti-measles	Pre-booster	163	92.6	87.5, 96.1	1595.8	1312.3, 1940.5	162	94.4	89.7, 97.4	1837.6	1529.9, 2207
≥150 mIU/ml	Post-booster	163	100	97.8, 100	2549.9	2236.6, 2907.2	161	99.4	96.6, 100	2690.1	2379.7, 3041
Anti-mumps	Pre-booster	154	82.5	75.5, 88.1	654.4	550.6, 777.8	158	80.4	73.3, 86.3	663.0	554.7, 792.5
≥231 U/ml	Post-booster	153	100	97.6, 100	2437.7	2159.5, 2751.6	158	100	97.7, 100	2251.3	2037.6, 2487.5
Anti-rubella	Pre-booster	163	100	97.8, 100	62.3	55.1, 70.5	162	100	97.7, 100	64.8	57.2, 73.3
≥4 IU/ml	Post-booster	163	100	97.8, 100	139.7	128.5, 151.9	162	100	97.7, 100	156.8	146.2, 168.1
Anti-rubella	Pre-booster	163	97.5	93.8, 99.3	62.3	55.1, 70.5	162	98.1	94.7, 99.6	64.8	57.2, 73.3
≥10 IU/ml	Post-booster	163	100	97.8, 100	139.7	128.5, 151.9	162	100	97.7, 100	156.8	146.2, 168.1

levels against diphtheria, tetanus, and poliovirus types 1–3 antigens (Table 3). The difference between pre- and post-immunisation antibody GMCs ranged from 16.4-fold for anti-tetanus antibodies to 38-fold for anti-poliovirus types 1–3 antibodies. All subjects apart from one in the DTPa-IPV group achieved anti-tetanus antibody concentrations of ≥1 IU/ml. The upper limit of the asymptotic two-sided 95% CI for the difference in seroprotection rates against diphtheria, tetanus and the three polio viruses were below the predefined clinical limit for non-inferiority.

Prior to immunisation, seropositivity rates to pertussis antigens were similar in both groups (Table 1) and ranged from at least 51.3% (95% CI 43.2, 59.2) for anti-PT to 100% (95% CI 97.7, 100) for anti-PRN. One month after booster immunisation, all subjects were seropositive for anti-FHA and anti-PRN antibodies and all but one subject who received

Table 2
Ratios of adjusted antibody GMCs 1 month after booster immunisation, with their 95% CIs (ATP immunogenicity cohort)

Antibody	Group DTPa-IPV + MMR		Group DTPa + IPV + MMR		Post immunisation GMC/T ratio DTPa + IPV + MMR over DTPa-IPV + MMR	
	N[a]	Adjusted GMC/T[b]	*N*[a]	Adjusted GMC/T[b]	Ratio	95% CI
Anti-diphtheria	159	6.220	150	5.859	0.942	0.796, 1.115
Anti-tetanus	159	8.222	150	6.644	0.808	0.714, 0.914
Anti-PT	159	112.71	148	128.34	1.139	0.950, 1.365[c]
Anti-FHA	159	360.29	148	429.72	1.193	1.030, 1.382[c]
Anti-PRN	159	703.80	150	676.11	0.961	0.819, 1.127[c]
Anti-poliovirus type 1	145	2985.66	142	3258.44	1.091	0.877, 1.358
Anti-poliovirus type 2	142	3012.87	143	3514.47	1.166	0.950, 1.433
Anti-poliovirus type 3	138	4898.58	137	4813.27	0.983	0.815, 1.184
Anti-measles	163	2657.54	161	2579.88	0.971	0.865, 1.090
Anti-mumps	153	2446.70	158	2243.26	0.917	0.808, 1.040
Anti-rubella	163	140.57	162	155.81	1.108	1.009, 1.217

Upper limit of 95% CI below the pre-specified clinical limit of non-inferiority (1.5) for GMC/T ratios. 95% CI for GMC/T ratios for other antigens were exploratory evaluations.

[a] *N*: Number of subjects with pre- and post-vaccination results.

[b] GMC/T: Geometric mean concentrations/titres adjusted for baseline concentration.

[c] Terms.

Table 3
Differences in seroprotection/vaccine response rates 1 month after booster vaccination, with asymptotic 95% CIs (ATP immunogenicity cohort)

Endpoints	Group DTPa-IPV + MMR				Group DTPa + IPV + MMR				Group difference DTPa + IPV + MMR minus DTPa-IPV + MMR[e]	
	N[a]	n[b]	%[c]	95% CI	N[a]	n[b]	%[c]	95% CI	Difference	95% CI
Anti-diphtheria ≥0.1 IU/ml	165	165	100	97.8, 100	159	159	100	97.7, 100	0.0	−2.4, 2.3[e]
Anti-tetanus ≥0.1 IU/ml	165	165	100	97.8, 100	159	159	100	97.7, 100	0.0	−2.3, 2.4[e]
Anti-PT VR[d]	159	157	98.7	95.5, 99.8	148	147	99.3	96.3, 100	0.6	−1.8, 3.1
Anti-FHA VR[d]	159	154	96.9	92.8, 99.0	148	147	99.3	96.3, 100	2.5	−0.2, 5.7
Anti-PRN VR[d]	159	156	98.1	94.6, 99.6	150	150	100	97.6, 100	1.9	0.1, 4.6
Anti-polio virus type 1 ≥8	156	156	100	97.7, 100	151	151	100	97.6, 100	0.0	−2.5, 2.4[e]
Anti-polio virus type 2 ≥8	153	153	100	97.6, 100	152	152	100	97.6, 100	0.0	−2.5, 2.4[e]
Anti-polio virus type 3 ≥8	148	148	100	97.5, 100	147	147	100	97.5, 100	0.0	−2.5, 2.5[e]

[a] Number of subjects with available results.
[b] Number of subjects with seroprotective antibody levels post immunisation or vaccine response (PT, FHA, PRN).
[c] Percentage of subjects with seroprotective antibody levels or vaccine response post-immunisation.
[d] VR: Vaccine response for PT, FHA and PRN (defined as appearance of antibodies in initially seronegative subjects or a two-fold increase in antibody concentration in initially seropositive subjects).
[e] Upper limit of 95% CI below the pre-specified clinical limit of non-inferiority (10%) for difference in seroprotection rates.

DTPa-IPV were seropositive for anti-PT antibodies. Vaccine response rates for all three pertussis antigens were similar in the two groups, with at least 96.9% of subjects receiving DTPa-IPV and 99.3% of subjects receiving DTPa + IPV showing a vaccine response to all three pertussis antigens (Table 3). Post-immunisation GMCs for anti-PT, anti-FHA and anti-PRN antibodies were similar in both groups with an increase from pre- to post-immunisation GMCs of at least 10.5-fold for pertussis antibodies (Tables 1 and 2). The upper limit of the 95% CI for the ratio of pertussis antibody GMCs as defined above was below the predefined clinical limit for non-inferiority.

Prior to immunisation >92% and >80% of subjects were seropositive for anti-measles and anti-mumps antibodies, respectively, and all subjects were seropositive for anti-rubella antibodies. Pre- and post-vaccination seropositivity rates and GMCs for anti-measles, anti-mumps and anti-rubella antibodies 1 month after the second MMR dose are shown in Table 1. Anti-measles, anti-mumps and anti-rubella GMCs were similar in both groups after immunisation. All subjects were seropositive to mumps and rubella and all subjects apart from one (in the DTPa + IPV group) were seropositive to measles.

3.3. Reactogenicity

The incidence and intensity of solicited local and general symptoms were similar in both groups (Tables 4a and 4b). Solicited general symptoms of Grade 3 intensity during the first 4 days post-immunisation were uncommon (reported for <3%) although Grade 3 general symptoms were reported for a slightly higher number of children who received the combined DTPa-IPV vaccine. Axillary temperature > 39 °C within 4 days after vaccination was observed in 1.8% (95%

Table 4a
Incidence of solicited local symptoms for individual vaccines during the 4-day follow-up period after immunisation (ATP safety cohort)

Symptom	Intensity	DTPa-IPV N = 171		DTPa N = 167		IPV N = 167		MMR[a] N = 171		MMR[b] N = 167	
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Pain	Any	137	80.1 (73.3, 85.8)	107	64.1 (56.3, 71.3)	87	52.1 (44.2, 59.9)	48	28.1 (21.5, 35.4)	61	36.5 (29.2, 44.3)
	Grade 3[c]	12	7.0 (3.7, 11.9)	6	3.6 (1.3, 7.7)	2	1.2 (0.1, 4.3)	1	0.6 (0.0, 3.2)	2	1.2 (0.1, 4.3)
Redness	Any	133	77.8 (70.8, 83.8)	129	77.2 (70.1, 83.4)	105	62.9 (55.1, 70.2)	40	23.4 (17.3, 30.5)	57	34.1 (27.0, 41.9)
	>20 mm	100	58.5 (50.7, 66.0)	104	62.3 (54.5, 69.6)	37	22.2 (16.1, 29.2)	4	2.3 (0.6, 5.9)	7	4.2 (1.7, 8.4)
	>50 mm	55	32.2 (25.2, 39.7)	69	41.3 (33.8, 49.2)	1	0.6 (0.0, 3.3)	1	0.6 (0.0, 3.2)	1	0.6 (0.0, 3.3)
Swelling	Any	103	60.2 (52.5, 67.6)	109	65.3 (57.5, 72.5)	69	41.3 (33.8, 49.2)	20	11.7 (7.3, 17.5)	28	16.8 (11.4, 23.3)
	>20 mm	73	42.7 (35.2, 50.5)	70	41.9 (34.3, 49.8)	13	7.8 (4.2, 12.9)	4	2.3 (0.6, 5.9)	6	3.6 (1.3, 7.7)
	>50 mm	41[d]	24.0 (17.8, 31.1)	47[e]	28.1 (21.5, 35.6)	0	0.0 (0, 2.2)	0	0.0 (0, 2.1)	1	0.6 (0, 3.3)

[a] MMR given concomitantly with DTPa-IPV.
[b] MMR given concomitantly with DTPa and IPV.
[c] Crying when the limb was moved or a spontaneously painful limb.
[d] Four subjects had swelling up to the shoulder joint, four subjects had swelling beyond the shoulder joint, two subjects had swelling which included both shoulder and elbow joints, one subject had swelling extending to the midpoint between the elbow and wrist.
[e] Six subjects had swelling of the shoulder joint, two subjects had swelling beyond the shoulder joint, two subjects had swelling including both shoulder and elbow joints.

Table 4b
Incidence of solicited general symptoms for simultaneously administered vaccines reported during the 15-day follow-up period after vaccination (ATP safety cohort)

Symptom	Intensity	DTPa-IPV + MMR, $N = 171$			DTPa + IPV + MMR, $N = 167$		
		n[a]	%	95% CI	n	%	95% CI
Fever[b]	≥37.5 °C	57	33.3	26.3, 40.9	65	38.9	31.5, 46.8
	>39.0 °C	7	4.1	1.7, 8.3	9	5.4	2.5, 10.0
	Related	54	31.6	24.7, 39.1	61	36	29.2, 44.3
Drowsiness	Any	40	23.4	17.3, 30.5	40	24.0	17.7, 31.2
	Grade 3[c]	5	2.9	1.0, 6.7	3	1.8	0.4, 5.2
Irritability	Any	45	26.3	19.9, 33.6	55	32.9	25.9, 40.6
	Grade 3[d]	2	1.2	0.1, 4.2	0	0.0	0.0, 2.2
Loss of appetite	Any	35	20.5	14.7, 27.3	40	24.0	17.7, 31.2
	Grade 3[e]	4	2.3	0.6, 5.9	3	1.8	0.4, 5.2
Rash	Any	9	5.3	2.4, 9.8	12	7.2	3.8, 12.2
	Related	5	2.9	1.0, 6.7	6	3.6	1.3, 7.7

[a] Number of subjects reporting a general symptom during the 15-day follow-up period after immunisation.
[b] Temperature was measured by the axillary route. Grade 3 fever >39.0 °C.
[c] Drowsiness that prevented normal everyday activity.
[d] Crying that could not be comforted or that prevented normal everyday activity.
[e] Subject did not eat at all.

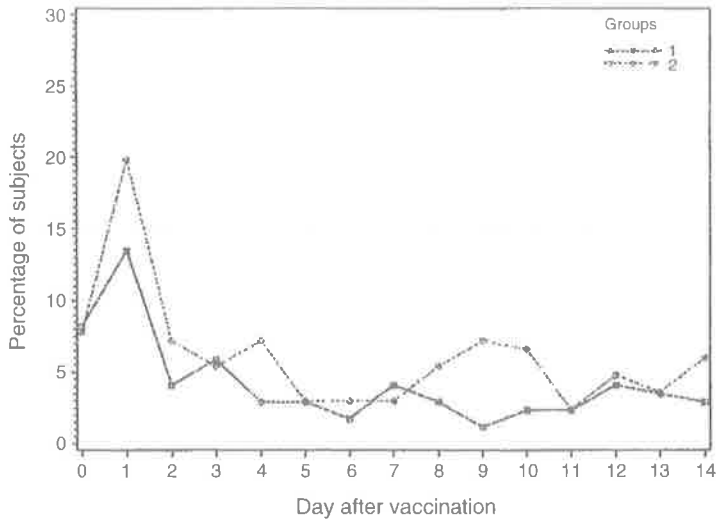
CI 0.4, 5.0) of children who received the DTPa-IPV vaccine compared to 0.6% (95% CI 0.0, 3.3) who received the vaccines administered separately.

Fig. 1 demonstrates the prevalence of fever from the day of immunisation to 14 days post-immunisation.

3.3.1. Large local reactions

There were no significant differences between groups in the incidence of large local reactions (Table 4a). Twenty five percent (24.6%, 95% CI 18.3, 31.7) of subjects ($n = 42$) who received DTPa-IPV vaccine compared to 29.3% (95% CI 22.6, 36.9) of subjects ($n = 49$) who received DTPa vaccine developed large local reactions. Almost one quarter of these reactions (23.8% of all large swelling reactions for the DTPa-IPV group and 20.4% for the DTPa group) involved swelling extending to an adjacent joint, which included swelling to the shoulder joint and both the shoulder and elbow joints in four subjects (Table 4a). One subject in the DTPa-IPV group developed swelling which extended below the elbow to approximately half the lower arm segment. However, no children developed swelling reactions involving the whole length of the arm.

A similar proportion of children experienced Grade 1/2 or 3 severity pain associated with swelling in both groups with a mean duration of 1.0 days for swelling >50 mm in the DTPa-IPV group and 2.3 days in the DTPa group (Table 5).



Group 1 received DTaP+IPV + MMR
Group 2 received DTaP+IPV + MMR

Fig. 1. Prevalence of any fever (axillary temperature ≥37.5 °C) by day (days 0–14).

Table 5
Incidence of redness and swelling by grades of pain during the 4 days (days 0–3) follow-up period per group (ATP cohort for safety)

Injection site reaction		Pain Grade 1 or 2				Pain Grade 3			
		n	%	95% CI	Mean duration (days)	*n*	%	95% CI	Mean duration (days)
Group 1 ($N = 171$)									
Redness	>0–≤20 mm	82	48.0	40.3, 55.7	1.7	8	4.7	2.0, 9.0	2.0
	>20–≤50 mm	64	37.4	30.2, 45.1	1.5	5	2.9	1.0, 6.7	1.6
	>50 mm	54	31.6	24.7, 39.1	2.0	6	3.5	1.3, 7.5	1.3
Swelling	>0–≤20 mm	65	38.0	30.7, 45.7	1.8	8	4.7	2.0, 9.0	1.8
	>20–≤50 mm	57	33.3	26.3, 40.9	1.8	4	2.3	0.6, 5.9	1.8
	>50 mm	39	22.8	16.7, 29.8	1.9	4	2.3	0.6, 5.9	1.0
Group 2 ($N = 167$)									
Redness	>0–≤20 mm	91	54.5	46.6, 62.2	2.6	6	3.6	1.3, 7.7	2.8
	>20–≤50 mm	70	41.9	34.3, 49.8	1.7	5	3.0	1.0, 6.8	1.4
	>50 mm	60	35.9	28.7, 43.7	2.2	6	3.6	1.3, 7.7	2.0
Swelling	>0–≤20 mm	74	44.3	36.6, 52.2	2.3	4	2.4	0.7, 6.0	2.3
	>20–≤50 mm	46	27.5	20.9, 35.0	1.8	3	1.8	0.4, 5.2	1.3
	>50 mm	41	24.6	18.2, 31.8	2.1	4	2.4	0.7, 6.0	2.3

Group 1: DTPa-IPV + MMR; Group 2: DTPa + IPV + MMR. Pain: Grade 1, minor reaction to touch; Grade 2, cried/protested on touch; Grade 3, cried when limb was moved/spontaneously painful. *N*: number of subjects having received the booster dose; *n*/%: number/percentage of subjects presenting at least one local symptom whatever the number of injections. For local symptoms and multiple injections, a symptom was counted once even if reported on multiple sites. 95% CI = Exact 95% confidence interval; LL = lower limit, UL = upper limit.

Only two subjects, both enrolled in the DTPa-IPV group, reported functional impairment of the limb severe enough to prevent normal everyday activities. One of these subjects had local swelling of 55 mm and experienced Grade 3 pain. The swelling started the day following vaccination and lasted 2 days. The second subject had diffuse swelling involving the tip of the shoulder with no further extension. Grade 3 pain was associated with the swelling, which started on the day of vaccination and lasted for 3 days. Severe impairment did not appear to be related to the extent of the reaction in this study. None of the four children with the most extensive reactions (involving shoulder and elbow) had severe functional impairment: only one had moderate impairment, the other three having either mild or no impairment. In addition, severe impairment in this study was not associated with a longer duration of swelling. Among the eight children with moderate or severe functional impairment, seven had recovered within 3 days and the eighth had recovered within 1 week.

All large swelling reactions commenced within 48 hours following immunisation, apart from one which commenced on the third day after administration of the booster. The large majority (80%) of the reactions had resolved within 4 days, 90% of the cases had resolved within 7 days and all recovered without sequelae.

In relation to solicited symptoms specific to MMR immunisation, rash assessed by the investigators as causally related to MMR vaccination was reported for 11 subjects in total, 2.9% (95% CI 1.0, 6.7) in the DTPa-IPV group and 3.6% (95% CI 1.3, 7.7) in the DTPa + IPV group (Table 4b). The incidence of local solicited symptoms at the site of the MMR injection was similar in both groups (Table 4a). One subject in the DTPa group developed swelling >50 mm at the site of the MMR immunisation (swelling diameter of 115 mm). One subject in the DTPa-IPV group developed salivary gland swelling 14 days following MMR immunisation. There were no reported cases of meningism.

There was only one serious adverse event (SAE) reported during the study period, which was assessed as possibly related to vaccination. The subject developed a high fever and headache 3 days post-vaccination with DTPa + IPV + MMR, and was later diagnosed with bronchitis.

4. Discussion

In order to maintain high immunisation coverage rates at a time when there are increasing numbers of vaccines available for protection of children against infectious disease, combination vaccines are becoming an important priority in vaccine development [23–25]. However, antigens cannot be combined indiscriminately as each combination may affect the immunogenicity of the individual components. This has been seen, for example, with many of the *Haemophilus influenzae* type b combination vaccines that have shown reduced immunogenicity for some antigen components [26,27].

This study assessed the safety and immunogenicity of a combination DTPa-IPV vaccine co-administered with MMR as a pre-school booster. The moderately low pre-booster seroprotective rates for diphtheria and pertussis toxoid support the need for a combined DTPa-IPV booster immunisation at pre-school age to provide long-term protection. The primary objective of the study was to demonstrate that the immunogenicity of the DTPa-IPV vaccine 1 month after vaccination is at least as good as that of DTPa and IPV administered separately when both groups received a separate concomitant

injection of MMR vaccine. All pre-defined non-inferiority criteria for meeting this objective were met.

Comparisons of the immune responses against all antigen components administered in the study vaccines, confirmed the similarity of the two study groups. Although many subjects had seroprotective/seropositive antibody concentrations/titres immediately prior to immunisation, marked increases in the GMC/GMT were observed, indicating a booster response induced by immunisation.

The secondary objective was to assess the safety and reactogenicity of the study vaccines administered in both groups and to assess the immunogenicity of MMR vaccine antigens 1 month after vaccination. This study provides immunogenicity and safety data for co-administration of a pre-school booster MMR vaccine with DTPa-IPV, which has not been available previously.

The immune responses to measles, mumps and rubella antigens administered concomitantly were similar within the two study groups and are consistent with or higher when compared with previously reported responses to a second dose of MMR vaccine [28]. Adverse events to MMR vaccine were similar in both groups and were present in this study in similar or reduced frequency to other literature reports [29]. The incidence of rash assessed as causally related following MMR vaccine co-administered with either DTPa-IPV or DTPa + IPV was less than 5%.

Reactogenicity events were reported similarly for both groups. The incidence of fever and other systemic symptoms was low and Grade 3 systemic symptoms occurred infrequently (<3% of subjects). Despite an improved safety profile in comparison to whole cell pertussis vaccines, acellular pertussis vaccines are known to cause an increase in large local reactions with the fourth and fifth booster doses [13–19]. The reported incidence of large local reactions in this study is consistent with results of previous studies [13–19]. A total of 32% of subjects reported redness >50 mm and 24% reported swelling >50 mm after DTPa-IPV vaccination. The local reactogenicity of the combined DTPa-IPV vaccine was not increased compared to DTPa vaccine in this study. In fact there was a higher (but not significant) reported incidence of redness and swelling >50 mm in the group that received DTPa separately (41% and 28%, respectively). Injection site erythema >50 mm has been reported in the literature in up to 50% of subjects and swelling reactions >50 mm in up to 48% of subjects following a fifth consecutive dose of a DTPa based vaccine [17–19,30]. In a trial in the USA up to 3% of children given two or more different acellular combination vaccines experienced entire limb swelling, although this did not occur in our study [18].

There does not appear to be any previously reported relationship between the extent or size of the swelling reaction and the amount of pain or limitation recorded. In this study, children who experienced the largest reactions did not report more Grade 3 pain and limitation of movement. Halperin observed that despite their larger size, local reactions to booster doses of an acellular pertussis combination vaccine have been less painful and limiting than those induced by consecutive regimens of whole cell pertussis combination vaccines [31]. Among children who received five consecutive doses of acellular pertussis vaccine, severe limb tenderness and limitation of movement were cited less frequently (2% and 0%, respectively) than in recipients of a fifth consecutive dose of whole cell pertussis vaccines (49% and 36%, respectively) [31]. Mixed schedules (four doses of DTPw followed by DTPa) have been reported to lead to a much lower incidence of local reactions than the full five dose DTPw series, with limb tenderness of 1.9% and 0.8%, respectively, and soreness of 18.6% and 4.0%.

In Australia most children in recent years have been primed with three doses of DTPa and OPV during the first year of life. At the time our study was conducted, children in Australia also received a fourth dose of DTPa at 18 months of age, and a fifth dose before school entry [32]. The Australian Standard Vaccination Schedule (ASVS, 2003) now includes a three-dose primary course and a fourth dose at 4–5 years of age given concomitantly with a booster dose of MMR vaccine [1]. Although the fourth dose at 18 months was removed from the schedule because of evidence of persisting efficacy of acellular pertussis vaccines against pertussis, it was also believed that reducing the number of DTPa vaccinations from five to four would be beneficial through the anticipated reduction in incidence of local reactions.

Safety monitoring of new vaccines is of prime importance in the continued assessment of vaccine safety [33]. Every new combination vaccine needs to be evaluated and approved as a new entity. Whether the removal of a fourth dose of DTPa at 18 months in the ASVS will in effect result in a reduction in the number of cases of extensive swelling reactions to the preschool DTPa combination vaccines, will only be determined by continued surveillance and reporting of adverse events.

In conclusion, we have demonstrated that the DTPa-IPV combination vaccine has a similar reactogenicity profile to DTPa and is immunogenic when administered to children as a booster and when administered concomitantly with MMR vaccine. It is expected that increased use of IPV in the form of combination vaccines will reduce the frequency of VAPP due to oral live poliovirus vaccine. The introduction of IPV containing combination vaccines will not only mean fewer injections for infants and children but also the opportunity to provide safer vaccines for children.

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ORIGINAL ARTICLE

DTPa-HBV-IPV vaccine for primary vaccination of infants

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Aim: Combined vaccines have an increasingly important role to play in delivering these antigens acceptably. We describe the immunogenicity and reactogenicity of a combined DTPa-HBV-IPV vaccine (diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated poliovirus (DTPa-HBV-IPV: *Infanrix penta*)) when administered for the primary vaccination of infants resulting from a study where the primary objective was to demonstrate non-inferiority of the immune response induced by DTPa-HBV-IPV using an industrial-scale IPV production process.

Methods: Three hundred and fourteen infants received primary immunisation with DTPa-HBV-IPV at 2, 4 and 6 months of age. Routine *Haemophilus influenzae* immunisation was performed at 2 and 4 months of age at a separate injection site. Blood samples were taken at 2 and 7 months of age. Reactogenicity was assessed using diary cards for 7 days after each dose.

Results: One month after the primary course, at least 98.9% of subjects achieved seroprotective antibody concentrations/titres against diphtheria, tetanus, hepatitis-B and polio types 1, 2 and 3. More than 97% had a vaccine response to pertussis antigens. The incidence of local injection site reactions after DTPa-HBV-IPV was similar to that for the *Haemophilus influenzae* vaccine site. General reactions of Grade 3 intensity were uncommon.

Conclusions: The DTPa-HBV-IPV vaccine is a new combination of vaccines previously available separately, with established effectiveness and safety profiles. Combined vaccines reduce storage requirements and minimise the number of injections required, thereby reducing distress for infants and parents. DTPa-HBV-IPV was immunogenic with an acceptable safety profile and could replace separate administration of DTPa, HBV and IPV vaccines in infants.

Key words: combined vaccine; DTPa-HBV-IPV vaccine; primary vaccination; schedule.

Key Points

1. Combined vaccines have an important role in administering multiple antigens in a single injection to infants.
2. In more than 95% of subjects, the DTPa-HBV-IPV vaccine produces seroprotective antibody concentrations against diphtheria, tetanus, hepatitis B and polio, and a vaccine response to pertussis antigens after primary vaccination.
3. The DTPa-HBV-IPV vaccine is well tolerated and injection site reactions are generally minor.

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Each year the number of diseases preventable by immunisation increases as new vaccines are introduced into immunisation schedules worldwide. Practical implications of additional newly available vaccines include increased storage requirements, more injections to deliver, and concerns about increased simultaneous injections and antigen load among parents and doctors.

In Australia, routine immunisation against *Haemophilus influenzae* (Hib), hepatitis B (HBV) and *Streptococcus pneumoniae* has been progressively added to the National Vaccination Schedule (NVS).[1] In addition, as of 1 November 2005, oral poliomyelitis vaccine (OPV) was replaced with injectable inactivated poliovirus vaccines (IPV). Without the availability of combination vaccines, these changes in the schedule would require infants receive up to five injections in a single visit, as experienced until recently by infants in the United States.[2,3] In order to reduce the inconvenience and distress associated with delivering multiple simultaneous injections, parents and doctors may elect to delay some vaccines until a later date, resulting in additional doctor visits with their associated costs, a risk of delayed protection, and a higher risk of children not completing the whole vaccination schedule.

Multivalent vaccines have a great deal to offer in reducing health-care costs associated with vaccine transport and storage,

reducing extra visits to doctors for separate immunisations and increasing the acceptability of vaccination by avoiding the need for multiple injections in young infants.

A combined DTPa-HBV-IPV vaccine (*Infanrix penta*, GlaxoSmithKline Biologicals (GSK), Belgium) has been licensed for use in Australia and the United States. Pre-licensure clinical trials in which 4480 subjects received vaccination with DTPa-HBV-IPV have been recently reviewed by Yeh *et al.*[3] In these studies, combined DTPa-HBV-IPV was shown to be as immunogenic and well-tolerated as separately administered vaccines. The DTPa-HBV-IPV vaccine contains the same antigens as in the currently licensed *Infanrix*-HepB, but with the addition of IPV. We report the immunogenicity and safety of the combined DTPa-HBV-IPV vaccine when administered for primary immunisation to Australian infants at 2, 4 and 6 months of age, at the same time as the Hib conjugate vaccine (Liquid *PedvaxHIB*, Merck and Co., Inc, Whitehouse Station, NJ, USA) at 2–4 months of age. The only previous reports of these vaccines given concomitantly were in German and Lithuanian infants at 3, 4, 5 months and 3, 4.5, 6 months of age, respectively.[4,5]

Methods

This was a Phase III randomised double-blind study (number 217744/077). The primary objective of the study was to evaluate the immune response and reactogenicity of two formulations of the DTPa-HBV-IPV vaccine that differed only in the scale of IPV manufacturing.

Healthy infants were enrolled at three centres in Australia: The Vaccine and Immunisation Research Group at Murdoch Children's Research Institute and The University of Melbourne, the Paediatric Trials Unit, Women's and Children's Hospital in Adelaide, and the Princess Margaret Hospital for Children in Perth. The trial was conducted according to the Declaration of Helsinki (South Africa, 1996) and Good Clinical Practice Guidelines with approval of each institution's ethics review committees. Written, informed consent was obtained from parents/guardians before enrolment.

Healthy infants between 8 and 12 weeks of age and born after a normal gestational period of 36–42 weeks were eligible to participate. All subjects had received a dose of HBV vaccine within 7 days of birth. Subjects were excluded from participation if they had a history of previous diphtheria, tetanus, pertussis, polio or Hib vaccination or disease, if they suffered a major congenital defect, immunodeficiency disorder, serious chronic illness, acute illness, neurological disease including previous seizures, or if they had previously received blood products. Subjects were not enrolled if they had a history of allergy likely to be exacerbated by vaccination with DTPa-HBV-IPV, required chronic medication of any type, or if there was planned use of any other investigational product during the study period.

Subjects were randomised using an Internet-based randomisation algorithm with a minimisation procedure stratified by centre, to receive either one of two DTPa-HBV-IPV vaccines at 2, 4 and 6 months of age. The vaccines were identical except that the IPV component had been manufactured in a either small- or large-scale process: new vaccines for evaluation in clinical trials are produced in small quantities (small scale). Industrial level production requires manufacturing on a much larger scale and clinical trials may be performed to confirm the comparability of the new large-scale process. Each subject received vaccine made by the same manufacturing process at each immunisation visit. Hib vaccine was co-administered as a separate injection in the contralateral thigh at 2 and 4 months of age.

Vaccines

The DTPa-HBV-IPV vaccine (*Infanrix penta*) was manufactured by GSK Biologicals, Belgium. Each dose (0.5 mL) of DTPa-HBV-IPV vaccine contained ≥30 IU diphtheria toxoid, ≥40 IU tetanus toxoid, 25 μg pertussis toxin (PT), 25 μg filamentous haemagglutinin (FHA), 8 μg pertactin (PRN), 10 μg recombinant HBV virus surface antigen (HBsAg), 40D, 8D and 32D antigen units of poliovirus types 1, 2 and 3, respectively. The Hib vaccine (Liquid *PedvaxHIB*) was manufactured by Merck and contained 7.5 μg Hib polyribosyl-ribitol-phosphate (PRP) conjugated to *Neisseria meningitidis* outer membrane protein. The DTPa-HBV-IPV vaccine was administered using a 23-gauge 25-mm needle into the left anterolateral thigh. Hib vaccine was administered into the right anterolateral thigh.

Assessment of immunogenicity

Blood samples were collected from all subjects before administration of the first vaccine and 1 month after the third primary vaccination. Samples were stored at −20°C until shipment to GSK's laboratory in Belgium.

Anti-diphtheria and anti-tetanus antibody concentrations were measured by enzyme-linked immunosorbent assay (ELISA) with an assay cut-off of 0.1 IU/mL. Anti-PT, anti-FHA and anti-PRN IgG antibody concentrations were measured by ELISA (cut-off of 5 EL.U/mL). Anti-HBV surface antibody (HBs) was measured by ELISA (AUSAB, Abbot Laboratories) according to the manufacturer's instructions. The assay cut-off was 10 mIU/mL. Antibodies against the three polio virus types were measured by a virus microneutralisation assay.[6] The lowest dilution tested was 1/8.

For diphtheria, tetanus, HBV and polio, concentrations/titres equal to or above the cut-off were considered to be indicative of seroprotection. As there is no established correlation of protection against pertussis, a vaccine response was defined as the appearance of antibodies in initially seronegative subjects (antibody concentration <5 EL.U/mL), or maintenance of antibody concentrations in subjects seropositive prior to vaccination.

The effectiveness of the licensed Hib vaccine employed in this study has been demonstrated in Australian children,[7] and the immunogenicity of this Hib vaccine when co-administered with DTPa-HBV-IPV has been demonstrated elsewhere.[5] It was considered highly unlikely that the manufacturing change under investigation would influence the response to Hib. Consequently, anti-PRP antibody concentrations were not measured in this study.

Assessment of reactogenicity

Reactogenicity was assessed using diary cards for 8 days (Days 0–7) after each vaccination. Local symptoms of pain, redness

and swelling at the site of injection, and general symptoms of drowsiness, fever (axillary temperature ≥37.5°C), irritability/fussiness, loss of appetite, restlessness (sleeping less than usual), sleepiness (sleeping more than usual) and vomiting were actively solicited. Parents were supplied with rulers to measure redness and swelling. Any symptom that was absent was graded as '0'. When symptoms occurred, the intensity was graded by the investigators on a 3-point scale where the most severe symptom was 'Grade 3' defined as any of: cries when limb is moved/spontaneously painful (pain); a diameter >20 mm (swelling and redness); axillary temperature >39.0°C (fever); crying or irritability that could not be comforted (irritability/fussiness); and preventing normal, everyday activities (all other symptoms).

Any symptoms occurring within 30 days of each vaccination were recorded. Serious adverse events (SAEs) were recorded during the entire study until 30 days after the last vaccination, and for an extended safety follow-up period of an additional 5 months after the last study visit. A SAE was defined as any untoward medical occurrence that resulted in death, was life-threatening, resulted in persistent or significant disability/incapacity or that required inpatient hospitalisation or prolongation of existing hospitalisation. In addition, an important medical event that may have jeopardised the patient or may have required intervention to prevent one of the other outcomes listed above was also considered serious.

Statistical analysis

Antibody seroprotection rates against diphtheria and tetanus toxoids, polio types 1, 2 and 3, and vaccine response rates to PT, FHA and PRN for the According to Protocol (ATP) cohort were calculated with exact 95% confidence interval (CI). Geometric mean antibody concentrations/titres (GMC/T) with 95% CI were calculated from the antilog of the mean of log-transformed values. Antibody concentrations below the assay cut-off were given an arbitrary value of half the cut-off for the purpose of GMC/T calculation.

The analysis of safety was performed on the ATP cohort for safety. The incidence of solicited local and general adverse events (any or Grade 3 intensity) was calculated with exact 95% CI. An enrolled cohort of 310 subjects was planned in order to meet the primary non-inferiority objectives of the study.

The primary objective of the study was to demonstrate non-inferiority of the immune response induced by DTPa-HBV-IPV using the large-scale IPV production process. Non-inferiority was concluded if 1 month after the third dose the 95% CI on the difference between groups in diphtheria, tetanus, HBV and polio seroprotection rates was less than 10%, and if the 90% CI on the GMC ratio for PT, FHA and PRN was less than 1.5.

Results

A total of 314 subjects were enrolled and vaccinated (156 received a 'small-scale' lot and 158 a 'large-scale' lot). Eleven subjects dropped out of the study: two due to SAEs (one subject with 'viral fever' considered by the investigator to be possibly related to vaccination, and one subject who developed complex partial seizures associated with a hereditary seizure disorder which was considered unrelated to vaccination), four subjects withdrew consent and five subjects migrated from the study areas or were lost to follow-up. Nine subjects were eliminated from the ATP analysis of safety for the following reasons: received previous vaccination (2 subjects), vaccine not administered according to protocol (6 subjects), and diary card not returned (1 subject). A further 26 subjects were eliminated from the ATP cohort for immunogenicity: non-compliance with intervals between vaccination (3 subjects), non-compliance with blood sampling schedule (13) and missing serological data (10). In total, 305 and 279 subjects contributed to the ATP analyses of safety and immunogenicity, respectively. The mean age of the total cohort at the first dose was 8.6 weeks (standard deviation 0.86 weeks, range 8–12 weeks) and 56.1% of subjects were male.

Non-inferiority of the immune response elicited by the DTPa-HBV-IPV vaccine manufactured using the large-scale IPV manufacturing process was demonstrated for diphtheria, tetanus, HBV, polio (the upper limit of the 95% CI on the group differences in seroprotection rate was below 10%), PT and FHA (the upper limit of the 90% CI on the GMC ratio was less than 1.5). For PRN, non-inferiority could not formally be concluded: the upper limit of the 90% CI (1.56) marginally exceeded the predefined limit of 1.5. However, anti-PRN antibody responses were consistent with those for which vaccine efficacy against pertussis has been demonstrated.[8,9] Therefore, for the purposes of this report, results have been pooled in order to provide an overview of the immunogenicity and reactogenicity of the DTPa-HBV-IPV vaccine in infants.

Immunogenicity

One month after completion of primary immunisation, more than 99% of subjects had seroprotective antibody concentrations against diphtheria, tetanus and polio types 1, 2 and 3, and 98.9% against HBV (Table 1). At least 97.2% had a vaccine response to pertussis antigens. There were many-fold increases in antibody GMC/T after primary immunisation for all vaccine antigens.

Reactogenicity

A total of 98.9% of subjects provided data for the analysis of safety. The majority of subjects (93%) reported the occurrence of at least one symptom during the first 8 days following immunisation; however, symptoms of 'Grade 3' intensity were uncommon (Table 2). Swelling >20 mm was the most commonly reported Grade 3 solicited local symptom and occurred at a similar rate at both the DTPa-HBV-IPV and Hib injection sites. The incidence of all solicited local symptoms fell with subsequent vaccine doses.

Irritability was the most commonly reported general symptom that occurred after each dose, followed by restlessness and sleeping more than usual. Solicited general symptoms of Grade 3 intensity occurred after <3.4% of doses, with the exception of irritability/fussiness which occurred after 7.2% of doses overall. Other than fever >39.0°C and vomiting, general symptoms reduced in incidence and intensity with successive doses.

Table 1 Seroprotection/vaccine response rates and GMC/T following primary vaccination with DTPa-HBV-IPV (ATP cohort for immunogenicity)

Antibody cut-off	Timing	*N*†	% SP/VR‡	95% CI		GMC/T	95% CI	
				LL	UL		LL	UL
Diphtheria	Pre	248	34.7	28.8	41.0	0.1	0.1	0.1
≥0.1 IU/mL	Post	279	99.6	98.0	100	1.6	1.4	1.8
Tetanus	Pre	248	85.5	80.5	89.6	0.4	0.4	0.5
≥0.1 IU/mL	Post	279	100	98.7	100	2.2	2.1	2.4
PT ≥ 5 EL.U/mL	Pre	247	34.0	28.1	40.3	3.9	3.6	4.2
VR	Post	247	98.8	96.5	99.7	64.8	60.1	69.8
FHA ≥ 5 EL.U/mL	Pre	247	67.2	61.0	73.0	8.7	7.6	10.0
VR	Post	247	97.2	94.2	98.9	274.9	254.4	297.0
PRN ≥ 5 EL.U/mL	Pre	248	29.0	23.5	35.1	4.0	3.6	4.4
VR	Post	247	99.6	97.8	100	125.0	112.6	138.7
HBs	Pre	216	15.3	10.8	20.8	8.4	6.9	10.1
≥10 mIU/mL	Post	264	98.9	96.7	99.8	1821.0	1544.2	2147.3
Polio 1	Pre	195	82.6	76.5	87.6	32.7	26.7	40.1
≥1:8	Post	264	99.6	97.9	100	513.2	441.7	596.2
Polio 2	Pre	201	77.1	70.7	82.7	17.7	15.0	20.8
≥1:8	Post	261	99.6	97.9	100	397.7	342.1	462.2
Polio 3	Pre	209	37.3	30.7	44.3	7.3	6.4	8.3
≥1:8	Post	261	100	98.6	100	1063.4	935.1	1209.3

†Number of subjects with available results. ‡Percent of subjects with seroprotective antibody concentrations/titres or a vaccine response for PT, FHA, PRN. VR (vaccine response) defined as: for initially seronegative subjects, post-vaccination antibody concentration ≥5 EL.U/mL; for initially seropositive subjects, antibody concentration at Post ≥1 fold of the pre-vaccination antibody concentration. CI, confidence interval; FHA, filamentous haemagglutinin; GMC/T, geometric mean antibody concentrations/titres; LL, lower limit; Post, 1 month following Dose 3; Pre, prior to vaccination; PRN, pertactin; PT, pertussis toxin; UL, upper limit.

Table 2 Incidence of fever and clinical events of Grade 3 intensity occurring after vaccination at 2, 4 and 6 months of age

	Dose 1 (2 months)		Dose 2 (4 months)		Dose 3 (6 months)†
	DTPa-HBV-IPV *n* = 305 %	Hib *n* = 305 %	DTPa-HBV-IPV *n* = 300 %	Hib *n* = 300 %	DTPa-HBV-IPV *n* = 300 %
Local					
Pain	4.3	3.6	4.7	3.7	0.7
Redness	4.6	4.3	3.0	3.0	3.0
Swelling	5.2	5.2	5.0	5.3	1.0
General					
Temperature ≥ 37.5°C	29.5		31.3		14.0
Temperature > 39.0°C	0.3		1.0		0.3
Diarrhoea	1.3		1.0		0.3
Irritability/fussiness	8.9		9.7		3.0
Loss of appetite	1.3		0.7		0.7
Restlessness	4.9		3.3		1.7
Sleeping more than usual	3.6		1.3		0.7
Vomiting	0.3		0.3		0.7

†Hib vaccine not administered at 6 months of age. *n*, number of subjects with at least one administered dose; %, percentage of subjects reporting the symptom at least once during the follow-up period.

Unsolicited adverse events occurred after 45.8% of doses during the first 30 days after each vaccination. The most commonly reported events were upper respiratory tract infection (after 10.5% of doses), teething (9.2%), rhinitis (8.3%) and 'other' injection site reaction (5.8%). Unsolicited symptoms of Grade 3 intensity were experienced after 6.3% of all doses, none of which were considered to be related to vaccination by the investigators.

Fourteen SAEs occurred during the study until 30 days after the last vaccination: bronchiolitis (2 cases), urinary tract infection (2 cases), rotavirus gastroenteritis (3 cases), other gastroenteritis (2 cases), infected foreskin (1 case), viral infection (2 cases), urticaria (1 case) and seizure disorder (1 case). Of these, hospitalisation of one subject because of 'viral fever' the day after Dose 1 was considered by the investigator to be related to immunisation and resulted in the subject withdrawing from the study. Development of complex partial seizures after Dose 2 in a subject with a family history of paroxysmal choreoathetosis also resulted in the subject withdrawing from the study, although the disorder was considered unrelated to immunisation by the investigator as seizures are a recognised part of this condition.

Fifteen additional SAEs were reported during the extended safety follow-up period from 1 month until 6 months after the last vaccination. These were: recurrent otitis media requiring insertion of grommets (4 cases), viral illness or bronchiolitis (5 cases, one with febrile convulsions), aspiration following elective surgery (1 case), pneumococcal bacteraemia (1 case), pneumonia (2 cases), asthma (1 case) and skull facture (1 case). None were considered by the investigator to have a causal relationship to vaccination.

Discussion

The DTPa-HBV-IPV vaccine (*Infanrix penta*) is a new combination of previously available and well-known vaccines with established efficacy and safety profiles. In Australia, acellular pertussis vaccines (DTPa) completely replaced the use of whole-cell pertussis preparations in 1999. The superior safety profile of acellular pertussis combination vaccines, compared with their whole-cell counterparts, has been well documented.[10,11] The HBV component of the combined DTPa-HBV-IPV vaccine is similar to *Engerix*-B, used extensively in Australia and also worldwide for decades. Inactivated polio vaccines were used in the 1950s before the development of live attenuated OPV and have been used exclusively for polio control by some countries since that time.[12] Use of IPV has the advantage of eliminating the small (1 case in 2.4 million doses[13]) but significant risk of vaccine-associated-paralytic-poliomyelitis associated with the use of OPV. IPV is now included in the NVS, with federal funding for delivery to all children provided since November 2005. The combined DTPa-HBV-IPV vaccine itself has been used for primary and booster vaccination of infants in Europe in combination with IIib vaccine (DTPa-HBV-IPV/Hib: *Infanrix hexa*) since 2000 and was licensed in the United States in 2002 under the trade name *Pediarix*, replacing separate administration of DTPa, HBV and IPV vaccines in that country.[3] The DTPa-HBV-IPV vaccine is currently administered as part of the NVS in the Northern Territory and Western Australia.

Published studies evaluating the DTPa-HBV-IPV vaccine have been reviewed recently by Yeh[3] and encompass six clinical trials in which more than 4000 infants received DTPa-HBV-IPV. The immune response to the combined vaccine has been demonstrated to be similar to that of separate administration of the component vaccines. Additionally, reactogenicity of the combined vaccine was not different to administration of separate injections.

We have shown that the DTPa-HBV-IPV vaccine, including vaccine manufactured by the larger-scale IPV manufacturing process, is highly immunogenic in Australian children, with the vast majority of subjects developing seroprotective antibody concentrations against diphtheria, tetanus, HBV and polio, and a vaccine response to pertussis antigens after primary vaccination. For antigens such as tetanus and polio types 1 and 2 where high levels of seroprotection were present before vaccination because of the presence of maternal antibody, many-fold increases in GMC/T after completion of the primary course indicate successful priming. A study of the co-administration of DTPa-HBV-IPV and Liquid *Pedvax Hib* showed no evidence of interference in the immune response to either vaccine when the vaccines were co-administered at separate injection sites.[5]

DTPa-HBV-IPV was safe and well-tolerated. Symptoms that occurred at the injection site were frequent but generally mild. The local reactogenicity in the combined DTPa-HBV-IPV group (including the small and large IPV manufacturing process cohorts) was similar to that of the licensed Liquid *PedvaxHib* given in the opposite limb at Doses 1 and 2, an observation made previously in German infants when given at 3, 4 and 5 months of age.[4] Irritability/fussiness of Grade 3 intensity was reported in up to 9.7% of subjects after each dose. Measurement of irritability is highly subjective and may be considered to be study dependant because of the impact of the definition used on the rate observed and the influence of culture on reporting practices. Nevertheless, the incidence of Grade 3 irritability observed following DTPa-HBV-IPV was less than previous studies of DTPa-based vaccines in Australian subjects, in which excessive irritability defined similarly as crying and could not be comforted at times or at all was reported in 16.5–21.5% following Dose 3 of a primary vaccination course.[14] Safety after completion of the primary vaccination course was assessed for an extended period with no evidence of long-term adverse effects related to vaccination. Although a control group that received separate injections of DTPa, HBV and IPV was not utilised in this study, previous randomised controlled trials have unequivocally demonstrated the comparable immunogenicity and safety of combined DTPa-HBV-IPV compared with separate injections.[4,15]

The arrival of multivalent combined vaccines has aroused concerns among some members of the public that the infant immune system may be overwhelmed by the number of antigens in vaccines. However, the immunologically normal infant responds to many thousands or tens of thousands of antigens at one time.[16] The acellular pertussis components in DTPa-HBV-IPV are three purified antigens, compared with thousands of antigens present in whole-cell formulations based on killed whole pertussis organisms. This means, in fact, that acellular pertussis vaccines contain many fewer antigens than were used previously in pertussis protection strategies. There is no evidence to date that immunisation with multiple antigens adversely impacts the overall immune status of the infant,[16] and the number of purified antigens found in multivalent combination vaccines does not pose a significant challenge to the enormous capacity of the immune system.

Combined vaccines minimise the number of required injections, thereby saving time in immunisation clinics, reducing

infant and parental distress, avoiding the need for additional consultation for deferred doses and thereby improving timeliness of vaccination[17,18] and reducing transport and storage costs of vaccines. Combined vaccines have an important role to play in providing an acceptable method of administering multiple antigens in a single injection to Australian infants.

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A novel combined *Haemophilus influenzae* type b-*Neisseria meningitidis* serogroups C and Y-tetanus-toxoid conjugate vaccine is immunogenic and induces immune memory when co-administered with DTPa-HBV-IPV and conjugate pneumococcal vaccines in infants☆

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Abstract

Immunogenicity and safety of a novel combined *Haemophilus influenzae* type b-*Neisseria meningitidis* serogroups C and Y-tetanus-toxoid conjugate vaccine (Hib-MenCY-TT) candidate was evaluated when co-administered with DTPa-HBV-IPV(*Pediarix*[TM3]) + PCV7(*Prevnar*[TM4]) at 2–4–6 months of age. Anti-PRP concentrations ≥1.0 μg/mL were observed in 92.9–98.7%, rSBA-MenC/Y titres ≥1:8 in >98%, rSBA-MenC/Y titres ≥1:128 in >95.8 and >89.9% subjects. PRP and MenC responses were similar to respective controls (*ActHIB*[TM5] and *Menjugate*[TM6]) including for antibody persistence. Response to co-administered vaccines was not impaired. Polysaccharide challenge (PRP, PSC, PSY at 11–14 months of age) evidenced immune memory was induced for Hib, MenC/Y conjugate components. The safety profile of Hib-MenCY-TT was similar to controls. Hib-MenCY-TT administered according to the current US Hib vaccine schedule has the potential to induce protective antibodies against Hib and meningococcal-CY disease in infants and toddlers.

Keywords: *Neisseria meningitidis* serogroup C; *Neisseria meningitidis* serogroup Y; Vaccine

Abbreviations: ATP, according-to-protocol; DTPa-HBV-IPV, diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus vaccine; DTPw, diphtheria, tetanus and whole-cell pertussis vaccine; CI, confidence interval; GMC/T, geometric mean concentration/titre; HBs, hepatitis B surface antigen; Hib, *Haemophilus influenzae* type b; Hib-MenCY-TT, combined *Haemophilus influenzae* type b-*Neisseria meningitidis* serogroups C/Y vaccine conjugated to tetanus-toxoid; MenC-CRM197, MenC conjugate vaccine conjugated to diphtheria toxoid mutant 197; PCV7, 7-valent conjugate pneumococcal vaccine; PRP, polyribosyl ribitol phosphate; PSC/Y, *Neisseria meningitidis* capsular polysaccharide serogroups C/Y; SAE, serious adverse event; rSBA, serum bactericidal activity using baby rabbit complement; TT, tetanus-toxoid.

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[3] *Pediarix* is the Trademark of the GlaxoSmithKline group of companies.
[4] *Prevnar* is the Trademark of Wyeth.
[5] *ActHIB* is the Trademark of Sanofi Pasteur.
[6] *Menjugate* is the Trademark of Novartis.

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1. Introduction

In the United States (US) there are between 2400 and 3000 cases of meningococcal disease each year, with an approximate incidence of 1.0/100,000 [1,2]. During the last decade the proportion of meningococcal disease due to serogroup Y has risen from 2% in 1990–1992 to 39 and 22% in the period between 1996–2001 and 2001–2005, respectively [2–5]. In the same periods serogroup C accounted for 31 and 26% of cases overall [2,5], making serogroups C and Y together the cause of approximately half of the cases of meningococcal disease in the US. Most of the remaining cases are caused by serogroup B meningococci. Elsewhere in the world, serogroup C is an important cause of disease although it became less common in those countries where a meningococcal C conjugate vaccine has been introduced [6].

In the US, the incidence of meningococcal disease peaks during the first year of life, where serogroups C and Y together account for approximately half of the cases [1]. It is estimated that a combined CY conjugate vaccine administered to infants would, over time, prevent 48% more meningococcal cases than a monovalent serogroup C vaccine [4]. The monovalent meningococcal serogroup C conjugate vaccines existing for use in infants in most developed countries are not available in the US. A MenCY conjugate vaccine would be optimally developed as a combination with existing vaccines for infants, to avoid additional injections in this age group. A licensed meningococcal ACWY conjugate vaccine was introduced in the routine immunization program in the US for 11–12-year-old children but is poorly immunogenic in infancy [7].

Three formulations of a novel combined *Haemophilus influenzae* type b-*N. meningitidis* serogroup C and Y vaccine conjugated to tetanus-toxoid (Hib-MenCY-TT) for the primary vaccination of infants were evaluated. To simultaneously evaluate possible co-administration effects, the study vaccine was administered concomitantly with routinely administered, licensed vaccines. Antibody persistence after primary vaccination was assessed at 11–14 months of age and, immune memory was assessed by immunization with 10 μg of each plain Hib, MenC and MenY polysaccharides.

2. Patients and methods

2.1. Study design

This randomized study was performed at three sites in Australia between March 2003 and August 2004 and was conducted according to Good Clinical Practice guidelines and the Declaration of Helsinki (South Africa). Protocols were approved by the ethics review committees of the participating centers. Written informed consent was obtained from parents/guardians prior to enrolment. Three different formulations of the Hib-MenCY-TT conjugate vaccine were evaluated. The MenC immune response was compared to that following administration of a licensed monovalent MenC conjugate vaccine and the Hib response to a licensed monovalent Hib vaccine.

2.2. Study procedures

Healthy infants 6–12 weeks of age and vaccinated against HBV at birth were eligible and randomly allocated to one of five study groups (Fig. 1). Subjects were excluded in case of: major congenital defects or serious chronic illness; immunodeficiency; previous/intercurrent vaccination or disease with study vaccine antigens; allergy to any component of the vaccine; use or planned use of other investigational or non-registered drugs/vaccines; receipt of blood products including immunoglobulin before or during the trial; neurologic disorders or seizures; acute disease at the time of enrolment.

The three Hib-MenCY-TT groups received concomitant vaccination with pentavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated poliovirus (DTPa-HBV-IPV) and 7-valent pneumococcal conjugate (PCV7) vaccines. The Control MenC group received MenC vaccine co-administered with Hib and DTPa-HBV-IPV. The Control Hib group received Hib vaccine co-administered with DTPa-HBV-IPV and PCV7 vaccines. All vaccines were administered intramuscularly at 2, 4 and 6 months of age in the anterolateral thigh as separate injections. The Hib-MenCY-TT and Hib vaccines were administered in the left thigh, the other vaccines in the right thigh at different injection sites. At 11–14 months of age (challenge phase), the subjects received 10 μg of plain polysaccharide polyribosyl ribitol phosphate (PRP) and 10 μg each of plain meningococcal polysaccharides C and Y (PSC, PSY; given as one fifth (1/5) dose of *N. meningitidis* $ACW^{135}Y$ polysaccharide vaccine). These vaccines were administered intramuscularly in opposite deltoids. At the end of the challenge phase licensed PCV7 (two doses) and MenC conjugate vaccines were offered to the Control MenC and Control Hib groups, respectively. All subjects were offered a booster dose of Hib conjugate vaccine at study conclusion in accordance with the Australian Standard Vaccination Schedule, which includes a booster dose at 12 months of age.

The different characteristics of the vaccines and the different vaccination schedules did not allow the study to be fully blinded with respect to the knowledge of the immunization and clinical parameters. However, the personnel who analyzed the data were blind to the vaccines received.

2.3. Vaccines

All vaccines except MenC, PCV7 and Hib were developed and manufactured by GlaxoSmithKline Biologicals, Rixensart, Belgium. Hib-MenCY-TT vaccines were conjugated to tetanus-toxoid (TT). The formulations tested were Hib-MenCY-TT 2.5/5/5 containing 2.5 μg of PRP and 5 μg each of MenC and MenY polysaccharides, Hib-MenCY-TT

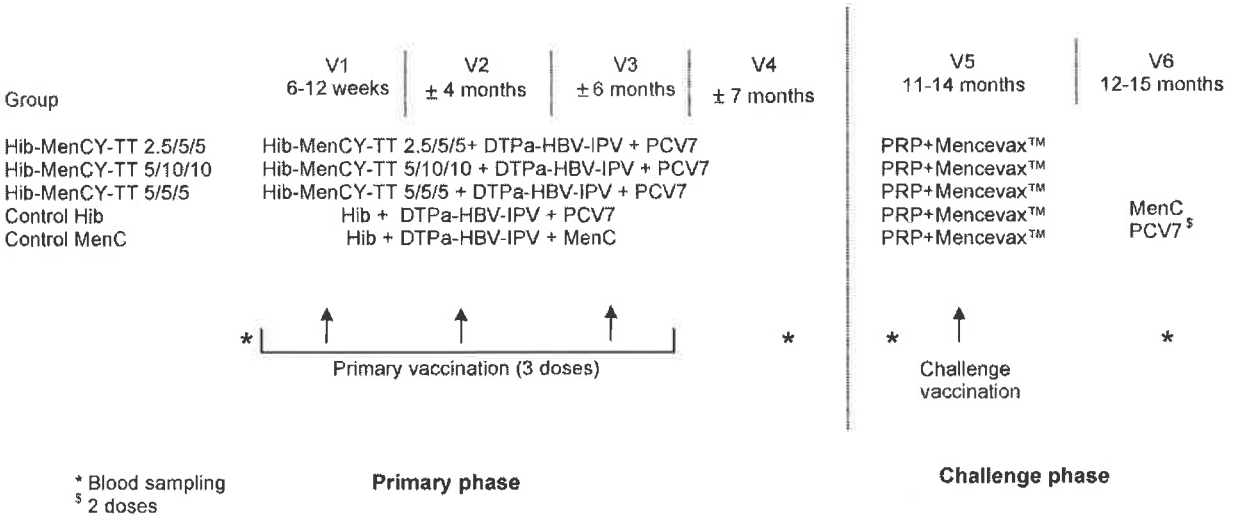

Fig. 1. Vaccination schedules.

5/10/10 with 5 μg of PRP and 10 μg each of MenC and MenY polysaccharides, and Hib-MenCY-TT 5/5/5 containing 5 μg of each polysaccharide, per 0.5 mL dose. The composition of DTPa-HBV-IPV vaccine (*Infanrix penta*™[7]/*Pediarix*™) has been described elsewhere [8]. *Mencevax*™ ACWY[8] contained 50 μg each of *N. meningitidis* polysaccharide serogroups A, C, W_{135} and Y. Plain PRP vaccine contained 10 μg PRP. The PCV7 vaccine (*Prevnar*™, Wyeth, Pearl River, NY, US) comprised pneumococcal polysaccharide serotypes 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to *Corynebacterium diphtheria* CRM197 protein, adsorbed onto aluminum phosphate. The licensed MenC vaccine (*Menjugate*™, Novartis, Emeryville, CA) contained 10 μg of MenC polysaccharide conjugated to CRM197 adsorbed onto aluminum phosphate. The licensed Hib conjugate vaccine (*ActHIB*™, Sanofi Pasteur, Lyon, France) contained 10 μg PRP conjugated to TT.

2.4. Assessment of antibody response

Four blood samples were collected from all subjects. Blinded serological analyses were performed at the laboratory of Dr. M. Pichichero (Department of Microbiology and Immunology's Labs, University of Rochester, NY, US) or at GlaxoSmithKline Biologicals, Rixensart, Belgium (meningococcal and pneumococcal antibody testing only).

Functional anti-MenC and MenY activity was measured by a serum bactericidal test using baby rabbit complement [9] (rSBA, assay cut-off at dilution 1:8). Specific anti-PSC and anti-PSY IgG were measured by ELISA (assay cut-off of 0.3 μg/mL) [10]. ELISA was also used to measure antibodies against PRP (assay cut-off of 0.15 μg/mL), diphtheria and tetanus-toxoids (0.1 IU/mL), pertussis antigens (5 EL.U/mL), and hepatitis B surface antigen (HBs, 10 mIU/mL). Antibodies against poliovirus types 1, 2 and 3 were determined by a virus micro-neutralization test (cut-off of 1:8 dilution) [11]. Pneumococcal serotype specific total IgG antibodies were measured by 22F inhibition ELISA [12] (assay cut-off of 0.05 μg/mL) [13].

The rSBA titer of 1:8 has been proposed as the correlate of protection for MenC; a threshold of 1:128 has also been used to describe immunization results [14,15]. For anti-PSC, a threshold of 2 μg/mL was also calculated as this may correlate with protection after the plain meningococcal C polysaccharide vaccine [16]. For diphtheria, tetanus, each polio type, Hib and hepatitis B, an antibody level at or exceeding assay cut-off was considered to be protective. For pneumococcal polysaccharide antibodies a threshold level of ≥0.2 μg/mL was considered [17]. For Hib, a concentration of 0.15 μg/mL was considered indicative of protection after conjugate immunization [18] and a concentration of 1 μg/mL was considered as indicative of long-term protection after immunization with plain polysaccharide [19,20].

2.5. Assessment of safety

Diary cards were used to record solicited local and general symptoms for 8 days after each vaccination (days 0–7). Other symptoms were recorded up to 30 days after each vaccine dose and serious adverse events (SAEs) were recorded during the entire study period.

2.6. Statistical methods

The first co-primary objective of the study was to evaluate the non-inferiority of the Hib-MenCY-TT vaccines compared to the Hib control in terms of percentage of subjects with anti-

[7] *Infanrix penta* is the Trademark of the GlaxoSmithKline group of companies.

[8] *Mencevax* ACWY is the Trademark of the GlaxoSmithKline group of companies.

PRP antibody concentrations ≥1.0 μg/mL by determining the upper limit of the standardized asymptotic 95% confidence interval (CI) of the difference between the control group and each of the three Hib-MenCY-TT formulations and assessing with which non-inferiority limit (delta) these results were compatible.

The second co-primary objective was to evaluate the immunogenicity of the different formulations in terms of rSBA-MenC and rSBA-MenY titres ≥1:8. Geometric mean antibody concentrations/titres (GMC/Ts) with 95% CIs and seropositivity/seroprotection rates with exact 95% CIs were calculated. Differences between the Hib-MenCY-TT and control groups were assessed by calculating standardized asymptotic 95% CIs on the difference between groups in seroprotection/seropositivity rates and the 95% CIs on the GMC/GMT ratio between groups. The Control MenC group served as the control for rSBA-MenC and anti-PSC IgG responses and the Control Hib group for all other antigens and for the assessment of safety. The control groups also served as MenY-unprimed controls for the challenge with meningococcal polysaccharides. Two vaccine groups were considered statistically significantly different if the 95% CI for the difference in rates between the two groups excluded zero, or if the 95% CI for the GMC/GMT ratio between groups excluded one.

The difference between the groups in the incidence of solicited symptoms was explored using the Fisher exact test. The according-to-protocol (ATP) cohorts for safety analysis included all subjects: who had received at least one dose of study vaccine/control according to their random assignment, for whom the administration site of study vaccine/control was known, who did not receive a vaccine not specified or forbidden in the protocol.

The ATP cohorts for immunogenicity analysis included all evaluable subjects (i.e. those meeting all eligibility criteria, complying with procedures defined in the protocol and with no elimination criteria during the study) from the ATP cohorts for safety for whom assay results for antibodies against at least one study vaccine antigen component 1 month after the third vaccine dose (for primary ATP cohort) or 1 month after at least one PRP, PSC or PSY vaccine dose (for challenge ATP cohort) were available.

The target enrolment was limited to 400 subjects in this feasibility study to provide at least 320 evaluable subjects (64 per group). Assuming that 93% seroprotection rates in each group was attained, 64 evaluable subjects per group ensured that the asymptotic 95% confidence for the difference in seroprotection rates between two groups was equal to [−8.8; 8.8%]. In addition, 64 evaluable subjects per group would provide a seropositivity rate of SBA-MenC or SBA-MenY with a 95% confidence interval equal to [89.7; 100%] if the observed rate was 95%.

3. Results

A total of 409 subjects were enrolled and randomized in the primary phase between March 2003 and February 2004 and 407 subjects received vaccine. Three hundred and ninety-four subjects participated in the challenge phase of the study from December 2003 to August 2004 (Fig. 2). All groups were comparable in terms of demographic characteristics: the mean age of the total vaccinated cohort at the time of the first vaccination and at the time of the booster vaccination was, respectively, 8.1 weeks ±1.5(standard deviation) weeks and 11.2 months ±0.5 month.

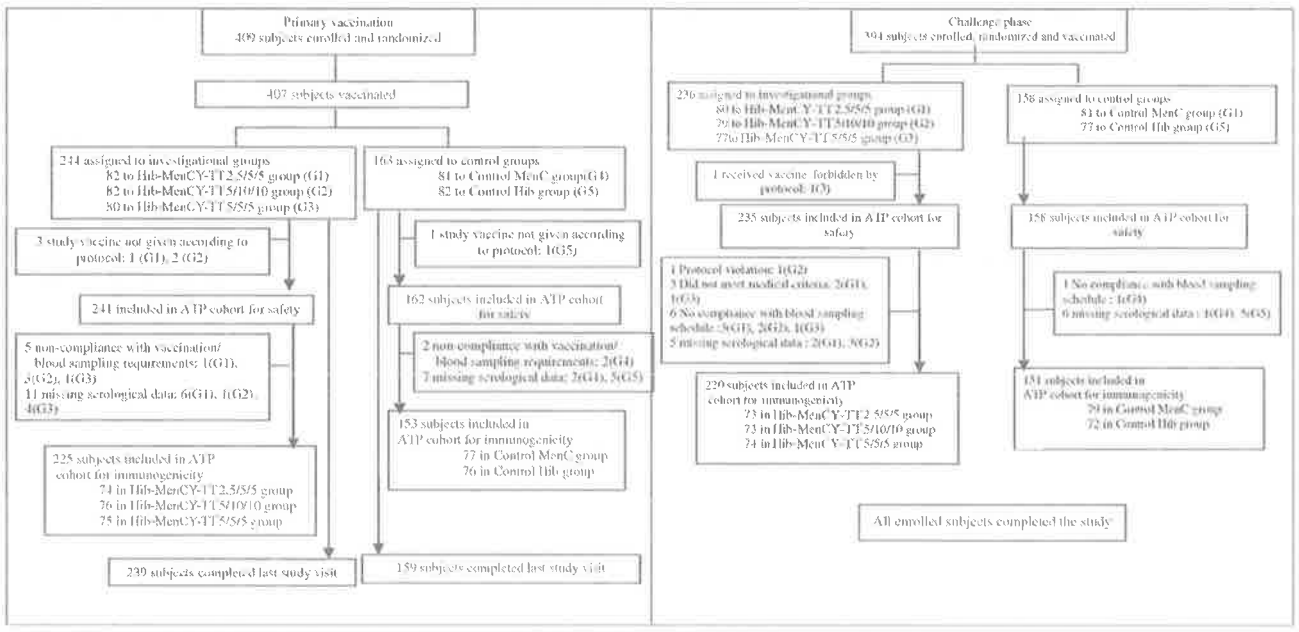

Fig. 2. Trial profile.

3.1. Immunogenicity

3.1.1. Response to PRP by ELISA

Anti-PRP seroprotection rates (≥0.15 μg/mL) and GMCs were similar in the Hib-MenCY-TT groups compared to the Control Hib group following primary vaccination and prior to polysaccharide challenge (Table 1; Fig. 3). Following primary vaccination, the proportion of subjects with anti-PRP antibody concentrations ≥1.0 μg/mL (Table 1) in Hib-MenCY-TT 2.5/5/5 and Hib-MenCY-TT 5/10/10 groups compared to the control was consistent with a non-inferiority limit (delta) of 5%, and a limit of 11% between the Hib-MenCY-TT 5/5/5 group and control (Table 2). After the polysaccharide challenge dose, statistically significantly more subjects in the Hib-MenCY-TT 2.5/5/5 and 5/10/10 groups achieved anti-PRP antibody concentrations ≥1.0 μg/mL than in the Control Hib group. Anti-PRP GMCs were statistically significantly higher in all three Hib-MenCY-TT groups compared to the Control Hib group.

3.1.2. Response to MenC

3.1.2.1. rSBA-MenC. After primary vaccination there were no statistically significant differences between the Hib-MenCY-TT groups and the Control MenC group in the proportion of subjects with rSBA-MenC titres ≥1:8 or ≥1:128 (Table 1). rSBA-MenC GMTs were statistically significantly higher in the Control MenC group after primary vaccination compared to each of the three Hib-MenCY-TT groups individually. However, prior to the polysaccharide challenge dose this difference was no longer evident (Fig. 3). Indeed, the persistence of rSBA-MenC was statistically significantly higher in the Hib-MenCY-TT 5/10/10 and 2.5/5/5 groups than in the Control MenC group in terms of titres ≥1:8 and ≥1:128, respectively, though there was no evidence for a difference in GMTs at that timepoint (Table 1).

After the polysaccharide challenge, the percentage of subjects with rSBA-MenC titres ≥1:128 was statistically significantly higher in the Hib-MenCY-TT 2.5/5/5 and 5/10/10 groups compared to the Control MenC group. rSBA-MenC GMTs were statistically significantly higher in the Hib-MenCY-TT 2.5/5/5 and 5/5/5 groups than in the Control MenC group.

3.1.2.2. Anti-PSC antibodies by ELISA. After primary vaccination, anti-PSC GMCs were statistically significantly lower in the Hib-MenCY-TT 5/5/5 group than the Control MenC group (Fig. 3). The two other Hib-MenCY-TT formulations were at the limit of statistical significance (the lower limit of the 95% CI on the GMC ratio was at 1.0). At 11–14 months of age, statistically significantly more subjects maintained anti-PSC antibody concentrations ≥2.0 μg/mL in the Hib-MenCY-TT 5/10/10 group versus the Control MenC group, although there was no evidence for a difference in GMCs at that timepoint (Fig. 3). After the polysaccharide challenge, anti-PSC GMCs were significantly higher in the Hib-MenCY-TT 2.5/5/5 and 5/5/5 groups than in the Control MenC group (Fig. 3).

3.1.3. Response to MenY

3.1.3.1. rSBA-MenY. After primary vaccination the proportion of subjects with rSBA-MenY titres ≥1:8 and ≥1:128, was substantially higher in the Hib-MenCY-TT-vaccinated

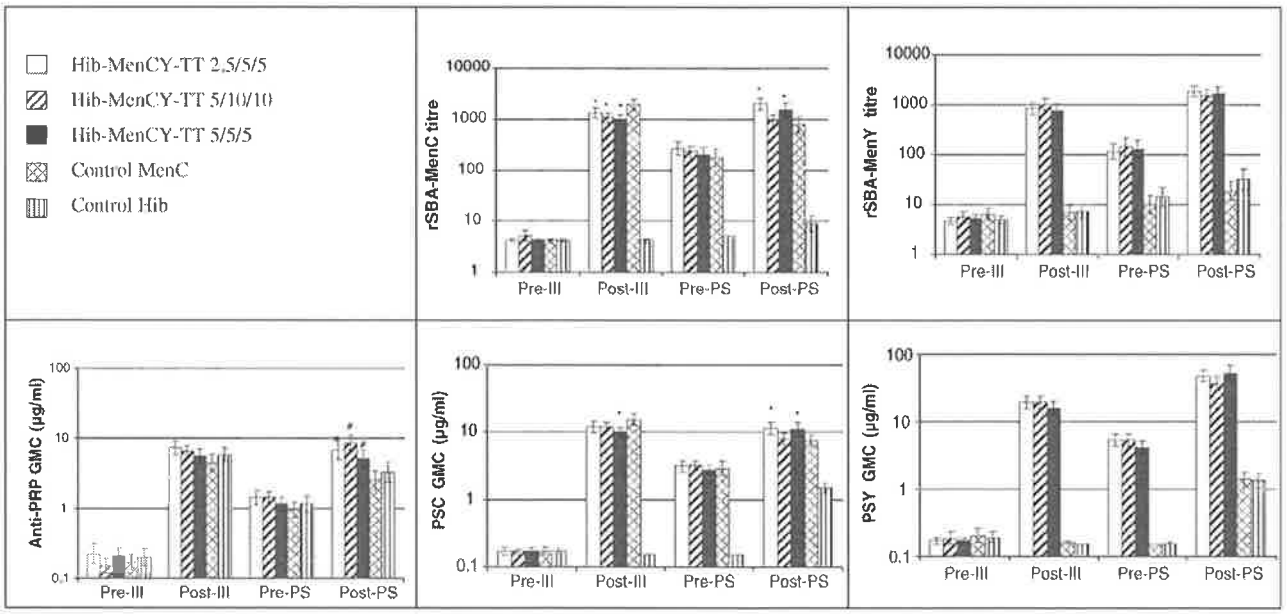

Fig. 3. Antibody GMC/Ts before and after primary vaccination, and before and after polysaccharide challenge at 11–14 months of age. Results from the ATP cohorts for immunogenicity from the primary and booster phases and ATP cohort for safety for antibody persistence prior to the challenge dose. Error bars = 95% CI; *or #, statistically significant difference compared to the control group: Control MenC or Control Hib, respectively (95% CI on the GMC ratio does not include "1"); pre-III and post-III, prior to and 1 month following primary vaccination; pre-PS and post-PS, at the time of and 1 month following plain polysaccharide challenge at 11–14 months of age.

Table 1
Percentage of subjects with antibody responses to PRP and MenC during the primary and polysaccharide challenge phases

Group	Timepoint		PRP				MenC							
			(μg/mL) %				rSBA (1/Dil) %				PSC (μg/mL) %			
		N	≥0.15	95% CI	≥1	95% CI	≥1:8	95% CI	≥1:128	95% CI	≥0.3	95% CI	≥2	95% CI
Hib-MenCY-TT 2.5/5/5	Pre-III	74	**42.6**	30.7;55.2	**10.3**	4.2;20.1	**2.9**	0.4;10.2	**0.0**	0.0;5.3	**9.3**	3.1;20.3	**1.9**	0.0;9.9
	Post-III	74	**100**	95.1;100	**97.3**	90.6;99.7	**100**	94.8;100	**98.6**	92.2;100	**100**	94.3;100	**98.4**	91.5;100
	Pre-PS	80	**100**	95.3;100	**66.2**	54.6;76.6	**97.4**	90.8;99.7	**78.9**[a]	68.1;87.5	**100**	94.9;100	**67.1**	54.9;77.9
	Post-PS	73	**100**	94.7;100	**98.5**[b]	92.1;100	**100**	94.8;100	**98.6**[c]	92.2;100	**100**	94.9;100	**94.4**	86.2;98.4
Hib-MenCY-TT 5/10/10	Pre-III	76	**52.9**	40.6;64.9	**14.3**	7.1;24.7	**9.1**	3.4;18.7	**1.5**	0.0;8.2	**9.3**	3.1;20.3	**0.0**	0.0;6.6
	Post-III	76	**100**	95.3;100	**98.7**	92.9;100	**100**	95.3;100	**97.4**	90.8;99.7	**100**	94.5;100	**100**	94.5;100
	Pre-PS	79	**98.7**	93.0;100	**59.7**	47.9;70.8	**98.7**[d]	92.9;100	**72.4**	60.9;82.0	**100**	95.1;100	**76.7**[e]	65.4;85.8
	Post-PS	73	**98.6**	92.6;100	**94.5**[f]	86.6;98.5	**100**	95.1;100	**97.3**[g]	90.5;99.7	**100**	94.9;100	**95.7**	88.0;99.1
Hib-MenCY-TT 5/5/5	Pre-III	75	**61.2**	48.5;72.9	**9.0**	3.4;18.5	**3.2**	0.4;11.0	**0.0**	0.0;5.7	**12.2**	4.6;24.8	**0.0**	0.0;7.3
	Post-III	75	**100**	94.9;100	**92.9**	84.1;97.6	**100**	95.0;100	**95.8**	88.3;99.1	**100**	94.1;100	**98.4**	91.2;100
	Pre-PS	76	**98.7**	92.8;100	**50.7**	38.9;62.4	**96.0**	88.8;99.2	**68.0**	56.2;78.3	**100**	94.3;100	**69.8**	57.0;80.8
	Post-PS	74	**100**	94.9;100	**91.5**	82.5;96.8	**100**	94.9;100	**94.4**	86.2;98.4	**100**	94.9;100	**93.0**	84.3;97.7
Control MenC	Pre-III	77	**47.8**	35.4;60.3	**9.0**	3.4;18.5	**4.5**	0.9;12.7	**0.0**	0.0;5.4	**8.9**	3.0;19.6	**0.0**	0.0;6.4
	Post-III	77	**98.6**	92.7;100	**89.2**	79.8;95.2	**100**	95.1;100	**98.6**	92.7;100	**100**	94.2;100	**100**	94.2;100
	Pre-PS	81	**93.6**	85.7;97.9	**51.3**	39.7;62.8	**90.8**	81.9;96.2	**64.5**	52.7;75.1	**100**	95.3;100	**57.9**	46.0;69.1
	Post-PS	79	**98.7**	93.1;100	**72.2**	60.9;81.7	**97.5**	91.2;99.7	**84.8**	75.0;91.9	**100**	95.3;100	**96.1**	88.9;99.2
Control Hib	Pre-III	76	**50.7**	38.7;62.6	**11.0**	4.9;20.5	**1.4**	0.0;7.7	**0.0**	0.0;5.1	**7.1**	2.0;17.3	**0.0**	0.0;6.4
	Post-III	76	**100**	95.1;100	**94.6**	86.7;98.5	**1.3**	0.0;7.1	**0.0**	0.0;4.7	**1.6**	0.0;8.5	**0.0**	0.0;5.7
	Pre-PS	77	**97.3**	90.6;99.7	**59.5**	47.4;70.7	**9.7**	4.0;19.0	**1.4**	0.0;7.5	**1.4**	0.0;7.6	**0.0**	0.0;5.1
	Post-PS	72	**98.6**	92.5;100	**80.6**	69.5;88.9	**27.3**	17.0;39.6	**9.1**	3.4;18.7	**97.2**	90.2;99.7	**39.4**	28.0;51.7

N, number of subjects in the ATP cohort for Immunogenicity primary phase, ATP cohort for safety challenge phase for pre-PS results and ATP cohort for immunogenicity for post-PS results; %, percentage of subjects with concentration above the specified cut-off; pre-III and post-III, prior to dose I and 1 month following dose 3 primary vaccination; pre-PS and post-PS, at the time of and 1 month following plain polysaccharide challenge at 11–14 months of age; PRP, polyribosyl ribitol phosphate; Men C, *N. meningitidis* serogroup C; PSC, *N. meningitidis* serogroup C polysaccharide rSBA, serum bactericidal activity using rabbit complement. Statistically significant differences: control group *minus* study group [95% CI for the difference in rates does not include "0"].

[a] Control MenC – Hib-MenCY-TT 2.5/5/5 [−28.4; −0.2].
[b] Control Hib – Hib-MenCY-TT 2.5/5/5 [−28.8; −9.0]
[c] Control MenC – Hib-MenCY-TT 2.5/5/5 [−23.5; −5.6].
[d] Control MenC – Hib-MenCY-TT 5/10/10 [−16.7; −1.0].
[e] Control MenC – Hib-MenCY-TT 5/10/10 [−33.1; −3.8].
[f] Control Hib – Hib-MenCY-TT 5/10/10 [−25.3; −3.5].
[g] Control MenC – Hib-MenCY-TT 5/10/10 [−22.4; −3.8].

Table 2
Difference between each of the three Hib-MenCY-TT groups and the control group in terms of percentage of subjects with anti-PRP concentration ≥1 μg/mL 1 month after the third vaccine dose (ATP cohort for immunogenicity)

Difference between groups	%	95% CI
Control Hib – Hib-MenCY-TT 2.5/5/5	−2.7	[−10.8;4.6]
Control Hib – Hib-MenCY-TT 5/10/10	−4.1	[−12.0;2.3]
Control Hib – Hib-MenCY-TT 5/5/5	1.7	[−7.0;11.0]

%, Percentage of subjects with anti-PRP concentration ≥1 μg/mL.

subjects, than in MenY-naïve subjects. Titers ≥1:128 persisted in >60% of children up to 11–14 months of age (Table 3; Fig. 3). The MenY GMT was also higher in the Hib-MenCY-TT groups. Plain polysaccharide challenge elicited increases in the percentage of subjects with rSBA-MenY titers ≥1:8 and ≥1:128 in all Hib-MenCY-TT groups and 11- to 16-fold increases in GMTs.

3.1.3.2. Anti-PSY antibodies by ELISA. One month after primary vaccination, anti-PSY antibody GMCs were of a similar order of magnitude in the three Hib-MenCY-TT groups and antibody concentrations ≥0.3 μg/mL were maintained in the majority of subjects until the polysaccharide challenge (Table 3; Fig. 3). One month after the plain polysaccharide challenge, the percentage of subjects with anti-PSY antibody concentrations ≥2.0 μg/mL was ≥98.6% in the three Hib-MenCY-TT groups compared with ≤36.4% in the MenY-naïve groups. Increases in GMC of 7.1 to 12.5 fold were also observed in the Hib-MenCY-TT-primed groups that were absent in the MenY-naïve groups.

3.1.4. Response to co-administered vaccines

With the exception of anti-tetanus antibody GMCs, there was no statistically significant difference between the Hib-MenCY-TT and Control Hib groups in terms of antibody seroprotection/seropositivity rates or GMC/Ts to DTPa-HBV-IPV antigens at either the post-primary or persistence timepoints (Table 4). Anti-tetanus antibody GMCs were higher at both timepoints in the Hib-MenCY-TT groups. One month after primary vaccination the anti-tetanus antibody GMC was 3.3, 3.8 and 3.4 IU/mL in groups Hib-MenCY-TT 2.5/5/5, Hib-MenCY-TT 5/10/10 and Hib-MenCY-TT 5/5/5, respectively, compared to 2.0 IU/mL in the Control Hib group. Prior to the polysaccharide challenge the anti-tetanus antibody GMC was 1.1 IU/mL, 1.3 IU/mL and 1.1 IU/mL in the respective Hib-MenCY-TT groups, compared to 0.8 IU/mL in Control Hib group. There was no statistically significant difference for any comparison between the three Hib-MenCY-TT groups and the Control Hib group in subjects with anti-pneumococcal antibodies ≥0.2 μg/mL or GMCs for any serotype (Table 5).

Table 3
Percentage of subjects with antibody responses to Men Y during the primary and polysaccharide challenge phases

Group	Timepoint		MenY							
		N	rSBA (1/Dil) %				PSY (μg/mL) %			
			≥1:8	95% CI	≥1:128	95% CI	≥0.3	95% CI	≥2	95% CI
Hib-MenCY-TT 2.5/5/5	Pre-III	74	**5.9**	1.6;14.4	**0.0**	0.0;5.3	**5.9**	1.2;16.2	**0.0**	0.0;7.0
	Post-III	74	**98.5**	92.0;100	**95.5**	87.5;99.1	**100**	94.6;100	**100**	94.6;100
	Pre-PS	80	**89.2**	79.8;95.2	**60.8**	48.8;72.0	**100**	95.3;100	**81.6**	71.0;89.5
	Post-PS	73	**100**	94.7;100	**100**	94.7;100	**100**	94.8;100	**100**	94.8;100
Hib-MenCY-TT 5/10/10	Pre-III	76	**10.8**	4.4;20.9	**3.1**	0.4;10.7	**7.8**	2.2;18.9	**3.9**	0.5;13.5
	Post-III	76	**100**	94.7;100	**97.1**	89.8;99.6	**100**	94.9;100	**98.6**	92.3 ;100
	Pre-PS	79	**81.1**	70.3;89.3	**67.6**	55.7;78.0	**100**	95.1;100	**86.5**	76.5 ;93.3
	Post-PS	73	**100**	94.9;100	**95.8**	88.1;99.1	**100**	94.8;100	**98.6**	92.4;100
Hib-MenCY-TT 5/5/5	Pre-III	75	**8.6**	2.9;19.0	**1.7**	0.0;9.2	**6.4**	1.3;17.5	**2.1**	0.1;11.6
	Post-III	75	**98.6**	92.2;100	**89.9**	80.2;95.8	**100**	95.0;100	**97.2**	90.3;99.7
	Pre-PS	76	**86.1**	75.9;93.1	**61.1**	48.9;72.4	**98.6**	92.6;100	**79.5**	68.4;88.0
	Post-PS	74	**98.6**	92.3;100	**98.6**	92.3;100	**100**	94.8;100	**98.6**	92.4;100
Control MenC	Pre-III	77	**14.3**	6.7;25.4	**7.9**	2.6;17.6	**12.5**	5.2;24.1	**3.6**	0.4;12.3
	Post-III	77	**14.7**	7.3;25.4	**8.8**	3.3;18.2	**3.0**	0.4;10.5	**0.0**	0.0;5.4
	Pre-PS	81	**23.6**	14.4;35.1	**13.9**	6.9;18.8	**0.0**	0.0;4.6	**0.0**	0.0;4.6
	Post-PS	79	**41.6**	53.4;60.4	**23.4**	14.5;34.4	**90.9**	82.2;96.3	**36.4**	25.7;48.1
Control Hib	Pre-III	76	**6.1**	1.7;14.8	**1.5**	0.0;8.2	**13.0**	5.4;24.9	**1.9**	0.0;9.9
	Post-III	76	**16.2**	8.7;26.6	**9.5**	3.9;18.5	**0.0**	0.0;5.2	**0.0**	0.0;5.2
	Pre-PS	77	**33.3**	22.7;45.4	**19.4**	11.1;30.5	**1.4**	0.0;7.6	**0.0**	0.0;5.1
	Post-PS	72	**53.5**	41.3;65.5	**38.0**	26.8;50.3	**90.1**	80.7;95.9	**33.8**	23.0;46.0

N, number of subjects in the ATP cohort for immunogenicity primary phase, ATP cohort for safety challenge phase for pre-PS results and ATP cohort for immunogenicity for post-PS results; %, percentage of subjects with concentration above the specified cut-off; pre-III and post-III, prior to and 1 month following primary vaccination; pre-PS and post-PS, at the time of and 1 month following plain polysaccharide challenge at 11–14 months of age; Men Y, *N. meningitidis* serogroup Y; PSY, *N. meningitidis* serogroup Y polysaccharide; rSBA, serum bactericidal activity.

Table 4
Antibody responses to DTPa-HBV-IPV after primary vaccination and prior to the polysaccharide challenge at 11–14 months of age

Group												
Antibody	Assay cut-off	Timepoint	Hib-MenCY-TT 2.5/5/5 $N=74(80)$		Hib-MenCY-TT 5/10/10 $N=76(79)$		Hib-MenCY-TT 5/5/5 $N=75(76)$		Control MenC $N=77(81)$		Control Hib $N=76(77)$	
			%	GMC/T	%	GMC/T	%	GMC/T	%	GMC/T	%	GMC/T
Diphtheria	0.1 IU/mL	Post-III	**100**	1.7	**100**	1.8	**100**	2.0	**100**	1.8	**100**	2.0
		Pre-PS	**97.4**	0.5	**96.2**	0.5	**96.0**	0.5	**92.5**	0.4	**100**	0.5
Tetanus	0.1 IU/mL	Post-III	**100**	3.3[a]	**100**	3.8[b]	**100**	3.4[c]	**100**	1.9	**100**	2.0
		Pre-PS	**100**	1.1	**100**	1.3	**98.7**	1.1	**97.5**	0.6	**100**	0.8
HBs	10 mIU/mL	Post-III	**98.6**	1769.2	**100**	1840.7	**100**	1652.6	**100**	1752.2	**98.7**	1609.7
		Pre-PS	**97.3**	452.3	**97.1**	490.5	**97.1**	452.9	**96.0**	390.5	**98.6**	534.9
PT	5 EL.U/mL	Post-III	**100**	55.1	**100**	55.2	**100**	53.7	**100**	49.7	**100**	54.8
		Pre-PS	**92.2**	11.6	**86.8**	11.6	**84.9**	9.9	**78.2**	8.2	**84.0**	9.8
FHA	5 EL.U/mL	Post-III	**100**	137.2	**100**	141.5	**100**	136.2	**100**	132.1	**100**	146.7
		Pre-PS	**98.7**	49.4	**98.7**	50.9	**96.0**	44.3	**100**	39.9	**100**	50.2
PRN	5 EL.U/mL	Post-III	**100**	128.3	**100**	120.7	**100**	106.2	**98.7**	112.9	**100**	137.8
		Pre-PS	**97.4**	36.2	**96.2**	31.8	**94.7**	27.3	**91.3**	27.2	**98.7**	35.8
Poliovirus 1	1:8	Post-III	**100**	669.6	**100**	476.8	**100**	574.7	**100**	454.0	**100**	517.6
		Pre-PS	**98.6**	175.8	**94.2**	120.6	**98.6**	162.4	**100**	116.4	**98.5**	166.4
Poliovirus 2	1:8	Post-III	**100**	533.8	**100**	369.8	**100**	408.0	**100**	348.9	**100**	368.0
		Pre-PS	**98.6**	139.8	**94.3**	94.1	**94.2**	110.1	**94.7**	89.6	**95.5**	88.3
Poliovirus 3	1:8	Post-III	**100**	1266.3	**100**	1034.8	**98.5**	1062.2	**98.5**	1084.9	**100**	945.5
		Pre-PS	**100**	348.4	**98.6**	263.8	**97.1**	250.9	**98.7**	264.0	**97.0**	203.9

N = number of subjects in the ATP cohorts for immunogenicity primary phase (ATP cohort for safety, challenge phase); %, percentage of subjects with concentration above the specified cut-off; post-III, 1 month following primary vaccination; pre-PS, at the time of the plain polysaccharide challenge at 11–14 months of age; GMC/T, geometric mean concentration/titre: GMC in μg/mL. Statistically significant differences: control group *divided by* study group [95% CI for the GMC/T ratio does not include "1"].

[a] Control Hib/Hib-MenCY-TT 2.5/5/5 [0.51; 0.74].

[b] Control Hib/Hib-MenCY-TT 5/10/10 [0.44; 0.64].

[c] Control Hib/Hib-MenCY-TT 5/5/5 [0.50; 0.72].

Table 5
Antibody response to PCV7 following primary vaccination and prior to the challenge dose at 11–14 months of age

Group											
Serotype	Timepoint	Hib-MenCY-TT 2.5/5/5 $N=74(80)$		Hib-MenCY-TT 5/10/10 $N=76(79)$		Hib-MenCY-TT 5/5/5 $N=75(76)$		Control MenC $N=77(81)$		Control Hib $N=76(77)$	
		≥0.2 μg/mL	GMC (μg/mL)	≥0.2 μg/mL	GMC (μg/mL)	≥0.2 μg/mL	GMC (μg/mL)	≥0.2 μg/mL	GMC (μg/mL)	≥ 0.2 μg/mL	GMC (μg/mL)
4	Post-III	100	2.101	100	2.049	100	2.023	1.7	0.027	100	2.062
	Pre-PS	92.9	0.495	88.2	0.528	92.4	0.508	1.4	0.026	82.8	0.45
6B	Post-III	85.3	1.06	91.4	1.079	81.2	0.834	1.6	0.027	86.2	0.879
	Pre-PS	65.2	0.307	65.1	0.307	79.0	0.292	0.0	0.026	67.7	0.308
9V	Post-III	100	3.102	97.2	2.363	100	2.823	1.6	0.028	98.5	2.651
	Pre-PS	91.7	0.818	90.0	0.721	98.2	0.933	3.2	0.03	100	0.881
14	Post-III	98.5	4.095	100	5.592	100	4.309	14.3	0.062	98.5	4.372
	Pre-PS	94.1	2.362	97.0	2.767	98.4	2.549	7.2	0.039	98.4	2.379
18C	Post-III	98.5	3.518	98.6	2.969	100	2.936	3.1	0.029	97.0	3.326
	Pre-PS	97.9	0.775	96.2	0.742	98.0	0.708	0.0	0.025	91.3	0.668
19F	Post-III	100	2.303	98.5	1.846	100	2.061	3.6	0.03	96.9	1.881
	Pre-PS	74.0	0.413	71.8	0.335	84.6	0.397	10.4	0.042	71.4	0.339
23F	Post-III	97.0	2.581	94.1	2.112	95.7	2.098	0.0	0.027	93.9	1.988
	Pre-PS	90.4	0.783	81.2	0.642	84.1	0.644	0.0	0.025	80.0	0.578

N, number of subjects in the ATP cohorts for Immunogenicity primary phase (ATP cohort for safety, challenge phase); %, percentage of subjects with concentration above the specified cut-off; post-III, 1 month following primary vaccination; pre-PS, at the time of the plain polysaccharide challenge at 11–14 months of age; GMC, geometric mean concentration.

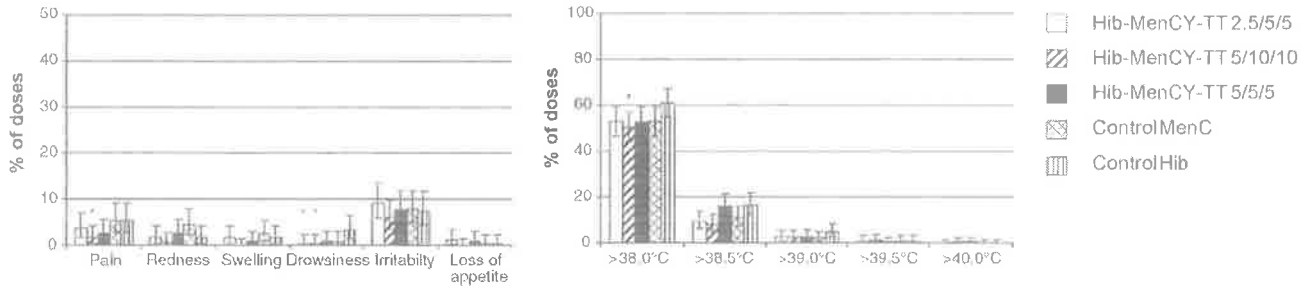

Fig. 4. Percentage of subjects with grade 3 solicited symptoms and any fever (axillary route) overall doses during 8-day follow-up after primary vaccination. Results from the total vaccinated cohort. Grade 3 defined as: pain; cries when limb is moved/spontaneously painful, redness and swelling; diameter >30 mm, loss of appetite; not eating at all, and for other adverse events; preventing normal, everyday activities and would cause the parents/ guardians to seek medical advice. Error bars = 95% CI, $^{*}p < 0.05$ Fisher exact test comparing group Control Hib.

3.2. Safety

The number of doses in the Hib-MenCY-TT 2.5/5/5 (0.4%) and Hib-MenCY-TT 5/10/10 groups (0.4%) which were followed by grade 3 drowsiness was statistically significantly less than in the Control Hib group (3.3%) ($p < 0.05$). Grade 3 pain also occurred less frequently ($p < 0.05$) in the Hib-MenCY-TT 5/10/10 group (1.6% of doses) compared to the Control Hib group (5.4%) (Fig. 4). No other statistically significant differences were noted in terms of grade 3 solicited symptoms. Fever >39.5 °C was reported by three subjects (1.2% of doses) or fewer in each group. The incidence of fever >38 °C was statistically significantly lower in the Hib-MenCY-TT 5/10/10 group compared to the Control Hib group ($p < 0.05$).

After primary vaccination, unsolicited symptoms of grade 3 intensity occurred in 12.2% to 18.3% of subjects vaccinated with a Hib-MenCY-TT vaccine, and in 17.1% of subjects in the Control Hib group, most of which were not related to vaccination. Twelve infants had unsolicited symptoms preventing daily activities and considered related to vaccination: 7 in the Hib-MenCY-TT groups (1 diarrhea, 1 constipation, 2 vomiting, one of them also feeling jittery, 1 upper respiratory tract infection, 1 eczema, 1 gastroenteritis), 2 in the MenC control group (1 gastroenteritis and 1 vomiting) and 3 in the Hib control group (2 vomiting and 1 upper respiratory tract infection). Most episodes lasted 1–3 days, except for respiratory tract infections (maximum duration of 23 days), one recipient with diarrhea (lasting 20 days) and one with eczema that was ongoing at study end.

Thirteen non-fatal SAEs occurred during the primary vaccination study: 6 in the 3 Hib-MenCY-TT groups and 7 in the control groups. Sixteen SAEs occurred from 1 month after primary vaccination until 1 month after the polysaccharide challenge. One child died at 6 months of age due to sudden infant death syndrome 88 days after receiving the first dose of Hib-MenCY-TT 5/5/5, but not having received the due second dose Hib-MenCY-TT 5/5/5. None of the SAEs were considered by the investigators to be related to vaccination.

4. Discussion

The novel Hib-MenCY-TT conjugate vaccine reported here is the first candidate vaccine that combines Hib antigen with conjugated MenC and MenY antigens. The goal in developing this product was to provide a vaccine for infants against two of the major serogroups contributing to meningococcal disease in infants in the US and other countries where MenY may become endemic in the future.

The MenY component in this vaccine resulted in the development of bactericidal antibodies in at least 98% of vaccinated infants. The Hib and MenC components of the Hib-MenCY-TT vaccines tested were as immunogenic as the monovalent vaccine currently available in Australia as determined by the proportion of subjects achieving anti-PRP antibody concentrations ≥ 0.15 and ≥ 1.0 μg/mL, and rSBA-MenC titres $\geq 1{:}8$ and $\geq 1{:}128$, after primary vaccination.

These results suggest that Hib-MenCY-TT administered according to the current US Hib vaccine schedule (2, 4, 6 and 12–15 months of age) has the potential to prevent Hib disease and an important component of meningococcal disease in infants and toddlers.

The availability of effective conjugate Hib vaccines has had a profound impact on the epidemiology of Hib disease [21]. The effectiveness of conjugate MenC vaccines in reducing MenC disease in both vaccinated and unvaccinated individuals has been conclusively demonstrated in the UK, where their use was widely implemented for epidemic control [22,23]. Recent papers from the UK suggest that a booster dose in late infancy or in the second year of life should be considered to ensure long-term sustained protection for both MenC [24] and Hib [25] and Hib and MenC conjugate booster doses are now part of the vaccination schedule in the UK.

It is reasonable to theorize that a combined conjugate vaccine against Hib, MenC and MenY will be similarly effective against the targeted diseases. The Hib-MenCY-TT vaccine reported in this article combines new antigens with an existing vaccine in the recommended US Pediatric Immunization Schedule, and is thus designed to require no additional injection or office visit.

Persistence and immune memory measured at a mean of 11 months of age allowed evaluation of immune memory by unconjugated polysaccharides in stringent conditions (considering that youngest children who are not primed by conjugate do not respond to polysaccharide). Persistence should be further evaluated when the candidate vaccine is given according to a routine immunization schedule such as 2–4–6 months with booster at 12–15 months as in the US.

There are no licensed MenY vaccines for infants, so in this study the MenY responses for the Hib-MenCY-TT combinations were compared with naïve controls. The MenY component conjugated to TT in the novel Hib-MenCY-TT vaccine induces functional bactericidal antibodies and immune memory in primed subjects, and is therefore likely to be protective against serogroup Y disease.

We observed that the significantly higher rSBA-MenC titres after vaccination with the licensed MenC vaccine (*Menjugate*™) did not translate to a higher proportion of subjects with rSBA-MenC levels of 1:8 or 1:128 either after primary vaccination or at the time of the polysaccharide challenge. On the contrary, significantly higher levels of rSBA-MenC persistence were seen in some Hib-MenCY-TT groups (2.5/5/5 and 5/10/10) that induced lower post-primary rSBA-MenC titres compared to the licensed product. In addition, in the Hib-MenCY-TT 2.5/5/5 and 5/10/10 groups, a higher proportion of subjects achieved rSBA-MenC titres $\geq$1:128 after polysaccharide challenge than subjects who received *Menjugate*™. Although interference with TT conjugated polysaccharide vaccines has been shown [26], we did not observe this in the current study with Hib-MenCY-TT.

All of the Hib-MenCY-TT combination vaccines tested contained lower amounts of PRP than currently licensed Hib vaccines. The ability of vaccines containing reduced amounts of PRP-TT to induce priming and immune memory that is equivalent to currently licensed products has now been well documented both for monovalent and combined Hib vaccines containing reduced PRP-TT, although these studies have either used DTPw for co-administration or in combination [27,28]. In agreement with recent data [29], we observed Hib responses in the three Hib-MenCY-TT groups containing reduced PRP concentrations (2.5 or 5 μg) that were comparable to those induced by the licensed Hib control vaccine (*ActHIB*™) containing 10 μg of PRP. The tetanus-toxoid carrier protein used to conjugate the two meningococcal components of the vaccine may also have enhanced the immunogenicity to the Hib vaccine as shown previously when such vaccines were co-administered simultaneously but at separate injection sites [30–32]. A significantly higher response was seen in the Hib-MenCY-TT 2.5/5/5 and 5/10/10 groups compared with *ActHIB*™, both in terms of the proportion of subjects who reached the 1.0 μg/mL cut-off and antibody GMCs after the polysaccharide challenge.

As for the MenC responses, the Hib responses demonstrate that the use of TT as a carrier did not lead to immune interference, in contrast to published studies with a multivalent pneumococcal TT based experimental conjugate vaccine [26,33], and appeared to lead to an enhanced response. Important parameters to be considered are the total content of TT, the number of conjugates using TT (in our case we are utilizing Hib-TT, MenC-TT and MenY-TT), the vaccine formulation including such factors as the use of adsorption and the co-administered DTPa or DTPw vaccine. New vaccine compositions/formulations such as Hib-MenCY-TT with the appropriate DTPa/Pw co-administrations and schedules need to be evaluated to ensure feasibility.

It is likely that a bivalent CY-vaccine is needed in the US for infants to achieve similar meningococcal disease reduction rates as those seen in the UK, Australia, and elsewhere following the introduction of serogroup C monovalent vaccines. The results presented in this study suggest that MenC and MenY conjugated to TT can be successfully combined with Hib conjugate vaccine containing a reduced amount of PRP without compromise in the immune response or reactogenicity profile of any component, and thereby avoiding additional injections in the already crowded US Pediatric Immunization Schedule. Results of this study also suggest that the Hib-MenCY-TT 2.5/5/5 vaccine can be administered in a 2–4–6-month schedule with other recommended vaccines, without immune interference, and is well tolerated. Subjects primed with the Hib-MenCY-TT 2.5/5/5 formulation showed consistently higher rSBA and antibody responses to MenC and PRP after polysaccharide challenge compared to those primed with commercially available monovalent Hib and MenC vaccines. In terms of the MenC response, similar or better persistence from the primary response up to the pre-booster timepoint was also observed. Results from a study where subjects were primed at an accelerated schedule (2–3–4 months of age) showed that the Hib-MenCY-TT 2.5/5/5 was the only formulation that did not show any statistically significant difference in the percentage of subjects with rSBA-MenC titers $\geq$1:128 compared to the Menjugate control [34]. The MenY component of the novel investigational vaccine is also immunogenic and induced robust priming and immune memory responses. The safety profile in the Hib-MenCY-TT groups was comparable to the control groups.

The combined Hib-MenCY-TT 2.5/5/5 vaccine is a novel investigational vaccine for infants and toddlers in the US designed to offer protection against meningococcal disease caused by serogroups C and Y and *H. influenzae* type b disease.

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Conflict of interest: These studies (study ID: 792014/001 and 792014/002, NCT number 00127855) were funded by GlaxoSmithKline Biologicals that was involved, in close collaboration with the investigators, in the study design, collection, analysis and interpretation of data, writing of the report and was part of the decision, in consultation with the investigators, to submit the paper for publication. Drs. Stephen Lambert and Don Roberton declare they do not have any conflict of interest with GlaxoSmithKline Biologicals. Drs. Helen Marshall, Peter Richmond and Terry Nolan declare they received travel grants from GlaxoSmithKline Biologicals. In addition, Dr. Terry Nolan declares he received consulting fees in the past 3 years, as an independent member of a Data Safety Monitoring Board for a study of another GlaxoSmithKline Biologicals vaccine. Drs. Dominique Boutriau, Jan Poolman and Catherine Streeton are employed by the GlaxoSmithKline group of companies and have stock ownership. In addition, Drs. Jan Poolman and Dominique Boutriau declare that they are inventor of patent applications.

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Chapter 2: New Respiratory Virus Vaccines

Respiratory viruses are the commonest cause of hospitalisation in children in Australia. Prevention by vaccination would result in a vast reduction in the number of paediatric admissions and health care costs associated with these ubiquitous infections. Influenza virus kills half a million people worldwide and 2,500 in Australia, annually. Over the past few centuries it has been associated with pandemics during which millions of people perished. Vaccines are available to prevent this infection, however require new components each year because of the chameleon nature of the virus. Constant genetic changes in influenza viruses mean that the vaccines' virus composition must be adjusted annually to include the most recent circulating influenza A(H3N2), A(H1N1) and influenza B viruses. During the past decades injectable, inactivated subunit vaccines have been used to induce systemic immunity to prevent influenza infection. However, alternative strategies have been identified which may offer several advantages over non-replicating vaccines. Intranasal administration of a live attenuated vaccine can induce both mucosal and systemic immunity and theoretically provide protection against both upper and lower respiratory tract infections.

Respiratory syncytial virus (RSV) is the major respiratory pathogen of young children and the major cause of lower respiratory tract infections (LRTI) in infants. Parainfluenza virus type 3 (PIV3) is a common cause of croup and LRTIs in children. Ideally, prevention of these respiratory infections with a combination vaccine given to infants would reduce the significant burden of disease in both developed and developing countries.

RSV subunit experimental vaccines have been developed and tested in clinical trials. Variability in antibody response to different RSV subunit vaccines and the likely requirement for annual vaccination based on waning immunity in the first year post vaccination, suggests application of the vaccine will be limited.

Intranasally administered, live attenuated vaccines may offer some advantages over subunit vaccines particularly for RSV-naïve infants and young children. As discussed above live attenuated vaccines administered intranasally are likely to confer both mucosal and systemic immunity, preventing both upper and lower respiratory tract infections.

Live attenuated intranasal influenza vaccine

5. Nolan T, Bernstein D, Block S, Hilty M, Keyserling H, Marchant C, **Marshall H**, Richmond P, Yogev R, Cordova J, Cho I, Mendelman P and for the LAIV Study Group. Safety and Immunogenicity of Concurrent Live Attenuated Influenza Vaccine With Measles-Mumps-Rubella and Varicella Vaccines in Infants 12 to 15 Months of Age. Pediatrics. 2008;121:508-516.

Live attenuated influenza vaccines (LAIV) have been developed and trialled in young children and shown to be immunogenic and safe. However, live vaccines can potentially affect the immune response to other live attenuated vaccines. MMR vaccine may interfere with the response to varicella vaccine if varicella vaccine is given in the first few weeks after MMR vaccine is administered. For this reason it is recommended that the two vaccines be given at the same immunisation encounter or four weeks apart. However, concomitant administration of live vaccines can also produce interference; concomitant administration of two live oral vaccines (polio vaccine and rotavirus vaccine) has been associated with a 40% reduction in sero-response rates to the live oral rotavirus vaccine. For this reason, it is important to establish the safety and immunogenicity of concomitantly administered investigational live virus vaccines.

In Paper 5 the safety, tolerability, and immunogenicity of a LAIV administered concurrently with MMR vaccine and varicella vaccine to healthy children 12 to 15 months of age were established. This multicentre, multi-country study showed that concomitant administration of live vaccines (MMR, varicella and LAIV) to children 12 to 15 months of age did not significantly affect the sero-response rates for MMR and varicella vaccines with simultaneous administration of LAIV. Strain specific seroconversion rates for each of the 3 LAIV vaccine strains were not altered by concomitant administration of MMR and varicella vaccines. In addition, concurrent administration of MMR vaccine, varicella vaccine, and intranasally administered LAIV was generally well tolerated. The study results confirmed that LAIV can be administered concomitantly to young children with MMR and varicella vaccines in routine clinical practice with no diminution of immunogenicity or safety. This is important because LAIV offers the potential benefits to young children of a broad immune response that includes both systemic and mucosal antibody responses and cellular immune responses, protection against strains that have antigenically "drifted" from the vaccine strains (as shown in previous studies) and LAV can be delivered without a needle.

The study results were presented at the Pediatric Academic Societies' Annual Meeting, 29 April – 2 May 2006, San Francisco, California. This vaccine, registered as "FluMist" is now licensed in the US with an expected filing for licensing in Australia within the next 12 months.

Live, Attenuated Parainfluenza Virus Vaccine

6. Belshe RB, Newman FK, Tsai TF, Karron RA, Reisinger K, Roberton D, **Marshall H**, Schwartz R, King J, Henderson FW, Rodriguez W, Severs JM, Wright PF, Keyserling H, Weinberg GA, Bromberg K, Loh R, Sly P, McIntyre P, Ziegler JB, Hackell J, Deatly A, Georgiu A, Paschalis M, Wu SL, Tatem JM, Murphy B, Anderson E. Phase 2 Evaluation of Parainfluenza Type 3 Cold Passage Mutant 45 Live Attenuated Vaccine in Healthy Children 6–18 Months Old. Journal of Infectious Diseases. 2004;189:462-470.

Human parainfluenza viruses are important causes of serious respiratory tract diseases in infants and young children under five years of age. PIV is an important cause of or co-factor in acute otitis media (AOM) in children. Type 3 PIV is of particular significance in that, in addition to causing croup and bronchitis, it ranks second only to RSV as a cause of bronchiolitis and pneumonia in infants less than 6 months of age. The virus causes severe disease throughout the first two years of life, and virtually all children have experienced primary PIV3 infections by three to four years of age. Overall, PIV3 is considered responsible for about 11% of hospitalisations for paediatric respiratory disease.

Past attempts to develop inactivated PIV3 vaccines showed that protection against disease was not induced, despite the development of serum antibodies after vaccination. Protection against PIV3 in humans is more likely to be achieved by the induction of both mucosal and humoral immunity. Protective mucosal and circulating antibodies should be induced most efficiently by delivery of a live attenuated virus vaccine to the mucosa of the respiratory tract.

The live attenuated virus vaccine evaluated in the study outlined in *Paper 6*, was derived from human wild-type PIV3 that was originally isolated from a child with a febrile respiratory illness. Several attenuated mutants of this strain were derived by passaging the virus numerous times in primary monkey kidney cells at sequentially lower temperatures that are suboptimal for PIV3 replication.

In previous Phase I studies PIV3-cp45 was generally well tolerated by all cohorts, with the exception that, in the seronegative cohort, otitis media was observed in 3 of 32 vaccine recipients and in none of 14 placebo recipients. Interpretation of the significance of this finding is uncertain because of the frequent acquisition of other intercurrent viral infections during the study period. The overall rates of upper respiratory tract infection (URTI) were very similar in the vaccine and placebo groups. We therefore undertook a Phase 2 study of PIV3-cp45 vaccine, administered intranasally to children 6–18 months to compare the safety profile of infants who received vaccine with that of placebo recipients and to assess the safety and immunogenicity of the vaccine. This Phase 2 study was therefore, specifically undertaken to assess the frequency of common signs and symptoms of acute otitis media and to obtain more precise estimates of the frequency of signs and symptoms of URTI that might be caused by vaccine virus replication.

Our results showed no difference in the incidence of adverse events (rhinorrhoea, cough or fever) between vaccine and placebo recipients. There was no increase in rhinorrhoea following vaccination with the PIV3-cp45 vaccine suggesting the vaccine is highly attenuated.

PIV3-cp45 vaccine was shown to be immunogenic, as seen in previous Phase I studies, with paired serum samples showed 84% of seronegative vaccine recipients developed a ≥4-fold increase in antibody titres. Amongst the seropositive subjects, the prevaccination titre did not increase after vaccination.

The results of this study suggest that this PIV3 vaccine should be evaluated for effectiveness in the prevention of respiratory illness and AOM in children.

I presented the results of this study at the 8th National Public Health Association of Australia Immunisation Conference in Melbourne, Victoria, Australia, May 16-17, 2002.

Live attenuated combination respiratory syncytial virus and parainfluenza virus type 3 vaccine

7. Belshe RB, Newman FK, Anderson EL, Wright PF, Karron RA, Tollefson S, Henderson FW, Meissner C, Madhi S, Roberton D, **Marshall H**, Loh R, Sly P, Murphy B, Tatem JM, Randolph V, Hackell J, Gruber W, Tsai TF. Evaluation of Combined Live, Attenuated Respiratory Syncytial Virus and Parainfluenza 3 Virus Vaccines in Infants and Young Children. Journal of Infectious Diseases. 2004;190:2096-2103.

Providing protection against a range of respiratory pathogens with one vaccine given intranasally would be of enormous convenience and benefit in reducing the burden of respiratory disease in children and adults. Experimental live attenuated respiratory virus vaccines being developed include different combinations of RSV, PIV3 and human metapneumovirus (hMPV), a recently discovered virus causing clinical symptoms similar to RSV infection.

Paper 7 describes the results of a study conducted to evaluate the safety, viral replication and immunogenicity of a live attenuated combination RSV-PIV3 vaccine in doubly seronegative (RSV, PIV3) children age 6-18 months of age. The objectives of this study were to describe the infection rate, magnitude, and duration of shedding of RSV and PIV3 after one dose of a combined RSV-PIV3 vaccine administered by intranasal delivery, to determine the safety of a combined RSV/PIV3 vaccine, to determine whether interference occurs when RSV and PIV3 are administered simultaneously, and to describe the antibody response as measured in serum and nasal wash specimens. The bivalent RSV-PIV3 vaccine, monovalent RSV vaccine, and monovalent PIV3 vaccine were compared in this multi-centre, international study.

The results of this study showed the majority of children in the bivalent vaccine group responded immunologically to both the RSV and the PIV3 vaccine component. However the study demonstrated very modest immunological interference by RSV with the PIV3 component in the bivalent group when compared to the response in the monovalent RSV and PIV3 groups. As a proof of principle study, the safety of the combined RSV-PIV3 vaccine could not be differentiated from those of either monovalent vaccine or placebo. Although no statistically significant differences were observed, it was noted that clinical cases of AOM occurred with increased frequency in the RSV and RSV/PIV3 groups. Confirmation of the significance of this observation will require a larger study due to the small sample size of this Phase I study and the high frequency of intercurrent virus infections in this young age group.

Genetic stability was confirmed in that the vaccine viruses retained their *temperature sensitive* phenotype despite multiple cycles of replication in young seronegative children. The multiple genetic changes introduced into this PIV3 and RSV vaccine provide a good means of safety to ensure that viruses with virulent phenotype will not emerge during replication in children.

Since the study was completed, significant progress has been made towards further attenuation of the RSV component, due to the safety concern (otitis media) identified in this study. A suitable RSV vaccine with greater attenuation is required, to be combined with PIV3 vaccine and evaluated for safety, infectivity, and efficacy of each component. The RSV component in this study interfered with PIV3, but not the reverse, suggesting that further attenuated derivatives might not interfere or interfere to a lesser degree and therefore work well in combination with PIV3.

I presented the study results at the 9th National Public Health Association of Australia Immunisation / 1st Asia Pacific Vaccine Preventable Diseases Conference in Cairns, Queensland, Australia, August 19 – 20, 2004.

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ARTICLE

Safety and Immunogenicity of Concurrent Administration of Live Attenuated Influenza Vaccine With Measles-Mumps-Rubella and Varicella Vaccines to Infants 12 to 15 Months of Age

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Financial Disclosure: Drs Nolan, Bernstein, Block, Hilty, Keyserling, Marchant, Marshall, Richmond, Yogev, and Mendelman and the LAIV Study Group participated in this research study, which was sponsored by MedImmune. The Murdoch Children's Research Institute has received research grant support from MedImmune. Dr Bernstein is a consultant for MedImmune and has received honoraria for speaking engagements on behalf of MedImmune. Dr Block has served as a consultant and speaker for and has received research grant support from MedImmune. Dr Marchant has received research and grant support from GlaxoSmithKline, MedImmune, and Merck, has served on the speakers bureaus for GlaxoSmithKline and Sanofi Pasteur, and has served as a consultant for GlaxoSmithKline, MedImmune, Merck, and Sanofi Pasteur. Dr Marshall has been the principal investigator for several studies sponsored by the pharmaceutical industry, including MedImmune. Iksung Cho and Julie Cordova are current and former employees of MedImmune, respectively. Dr Mendelman is a former employee of and current consultant for MedImmune.

What's Known on This Subject

Childhood vaccines are often administered concurrently. There is a theoretical possibility that components of one vaccine may alter immune responses to another. The concurrent use of live attenuated influenza virus vaccine with other live vaccines for children has not been investigated.

What This Study Adds

Intranasally administered live attenuated influenza virus vaccine can be administered concomitantly with measles-mumps-rubella and varicella vaccines to young children in routine clinical practice without reducing the immunogenicity or safety of any of the vaccines.

ABSTRACT

OBJECTIVE. This study evaluated the safety, tolerability, and immunogenicity of live attenuated influenza vaccine administered concurrently with measles-mumps-rubella vaccine and varicella vaccine to healthy children 12 to 15 months of age.

METHODS. Children were assigned randomly to receive (1) measles-mumps-rubella vaccine, varicella vaccine, and intranasal placebo on day 0, followed by 1 dose of live attenuated influenza vaccine on days 42 and 72; (2) measles-mumps-rubella, varicella, and live attenuated influenza vaccines on day 0, followed by a second dose of live attenuated influenza vaccine on day 42 and intranasally administered placebo on day 72; or (3) 1 dose of live attenuated influenza vaccine on days 0 and 42, followed by measles-mumps-rubella and varicella vaccines on day 72. Serum samples were collected before vaccination on days 0, 42, and 72. Reactogenicity events and adverse events were collected through day 41 after concurrent vaccinations and through day 10 after administration of live attenuated influenza vaccine or placebo alone.

RESULTS. Among 1245 (99.5%) evaluable children, seroresponse rates and geometric mean titers for measles-mumps-rubella vaccine and varicella vaccine were similar with concurrent administration of live attenuated influenza vaccine or placebo (seroresponse rates of ≥96% for measles-mumps-rubella vaccine and ≥82% for varicella vaccine in both groups). Hemagglutinin-inhibiting antibody geometric mean titers and seroconversion rates to influenza strains in live attenuated influenza virus vaccine were similar after the vaccine was administered alone (seroconversion rates of 98%, 92%, and 44% for H3, B, and H1 strains, respectively) or with measles-mumps-rubella and varicella vaccines (seroconversion rates of 98%, 96%, and 43%). The incidences of reactogenicity events and adverse events were similar among treatment groups.

CONCLUSIONS. Concurrent administration of live attenuated influenza vaccine with measles-mumps-rubella vaccine and varicella vaccine provided equivalent immunogenicity, compared with separate administration, and was well tolerated.

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This trial has been registered at www.clinicaltrials.gov (identifier NCT00192491).

Key Words

live attenuated influenza virus vaccine, varicella vaccine, measles-mumps-rubella vaccine, immunogenicity, concurrent vaccinations, children

Abbreviations

AE—adverse event
CI—confidence interval
GMT—geometric mean titer
HAI—hemagglutination-inhibiting
LAIV—live attenuated influenza vaccine
MMR—measles-mumps-rubella
RE—reactogenicity event

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THE ADVISORY COMMITTEE on Immunization Practices currently recommends that healthy children receive >25 vaccine doses during their first 2 years to prevent infections, including influenza.[1] This schedule frequently necessitates concurrent administration of vaccines.

Although seroresponse rates and adverse reactions after administration of combinations of live attenuated vaccines are similar to those observed with separate administration,[2] immunogenicity, safety, and lack of interference with concurrent administration of multiple live vaccines must be established. Because of this theoretical risk, the Centers for Disease Control and Prevention recommends that live vaccines that are not administered concurrently be administered at intervals of >4 weeks.[2] Currently recommended vaccines for infants and young children that use live attenuated viruses as the immunogens include measles, mumps, rubella, varicella (frequently given as a combined product), and rotavirus vaccines.

Live attenuated influenza vaccine (LAIV, FluMist; MedImmune, Gaithersburg, MD) is currently approved for healthy children ≥2 years of age. Several studies have established that the frozen LAIV (and the recently licensed, refrigerator-stable formulation, referred to as cold-adapted influenza vaccine, trivalent) is immunogenic, efficacious, and well tolerated in young children.[3–9] It is important to demonstrate that concurrent administration of LAIV does not adversely affect the immune response to LAIV or other pediatric live vaccines. The objective of this study was to evaluate the safety, tolerability, and immunogenicity of concurrent administration of LAIV with measles-mumps-rubella (MMR) and varicella vaccines in healthy infants 12 to 15 months of age.

METHODS

Study Design

This randomized, placebo-controlled trial was conducted over 2 study seasons at 44 sites in the United States and 3 sites in Australia. To avoid potentially confounding factors in the interpretation of immune responses, children were enrolled outside periods of peak influenza activity in their respective regions. Study seasons in the United States were May through October, 2001 and 2002, and those in Australia were November 2000 through May 2001 and November 2001 through May 2002.

Eligible children were prospectively assigned randomly, in a 1:1:1 ratio, to 1 of 3 groups, as follows: MMR/varicella group, concurrent administration of MMR vaccine, varicella vaccine, and intranasal placebo treatment on day 0, followed by a single dose of LAIV on days 42 and 72; MMR/varicella/LAIV group, concurrent administration of MMR, varicella, and LAIV vaccines on day 0, followed by a second dose of LAIV on day 42 and intranasal placebo treatment on day 72; LAIV group, a single dose of LAIV on days 0 and 42, followed by concurrent dosing with MMR and varicella vaccines on day 72. Randomization was stratified according to season and site by using a block size of 6 and was achieved with a predefined randomization schedule that assigned a treatment group to each unique participant number. Each participant was assigned a unique participant number that was maintained throughout the trial. The MMR/varicella and MMR/varicella/LAIV groups were double-blinded with respect to treatment for the duration of the study. Because no injectable placebo treatments were used, treatment assignment to the LAIV group was unblinded after randomization.

The study was conducted in accordance with the Declaration of Helsinki, the US Code of Federal Regulations governing the protection of human subjects, and the International Conference on Harmonisation Guidance for Good Clinical Practice. The study protocol and informed consent documents were approved by the institutional review board or independent ethics committee of each site. Written informed consent was obtained from each subject's parent or legal guardian.

Study Participants

Eligible subjects were children 12 to 15 months of age who were in good health (determined by medical history and physical examination) and up to date with the primary series of recommended vaccines (according to standard clinic practice and local vaccine availability). Exclusion criteria included previous vaccination against or diagnoses of measles, mumps, rubella, or varicella; hypersensitivity to egg, egg protein, or any component of the study vaccines or placebo treatment; known or suspected immunosuppression or immunosuppressed household member; acute febrile (>100.0°F oral) illness or clinically significant upper respiratory illness within 72 hours before enrollment; receipt of aspirin (acetylsalicylic acid) or aspirin-containing products in the month before enrollment; receipt of any intranasally administered medication within 2 weeks before enrollment; receipt of any live virus vaccine within 1 month before enrollment through 30 days after the final visit; receipt of any inactivated vaccine within 2 weeks before enrollment through 30 days after the final visit; receipt of any blood product within 3 months before vaccination; and history of ≥2 episodes of medically attended wheezing or medically attended wheezing illness or bronchodilator medication use within 4 weeks before enrollment.

Vaccines

LAIV vaccine (MedImmune Vaccines, Mountain View, CA) was supplied in intranasal sprayers with a total volume of 0.5 mL, containing allantoic fluid stabilized with sucrose/phosphate/glutamate and ~10^7 median tissue culture infectious doses of each of the 3 attenuated vaccine strains grown in pathogen-free chicken eggs, that is, A/New Caledonia/20/99 (H1N1), A/Sydney/05/97 (H3N2), and B/Yamanashi/166/90. Vaccine was stored frozen at −15°C or below until just before intranasal administration (~0.25 mL into each nostril). Excipient placebo was supplied in intranasal sprayers with a total volume of 0.5 mL, containing allantoic fluid from pathogen-free eggs, stabilized with sucrose/phosphate/glutamate. MMR vaccine (M-M-RII; Merck, Whitehouse

Station, NJ) and varicella virus vaccine (Varivax; Merck) were supplied as single-dose vials of lyophilized vaccine.[10,11]

Study Evaluations

The primary objective was to compare the immune responses to measles, mumps, rubella, and varicella antigens in the MMR/varicella/LAIV and MMR/varicella groups and to compare the immune responses to the 3 strains of influenza (A/H1N1, A/H3N2, and B) in the MMR/varicella/LAIV and LAIV groups. Serum samples were obtained before vaccination on day 0 and on day 42 (for MMR and varicella vaccine responses) and day 72 (for LAIV responses).

Immunogenicity to mumps, measles, rubella, and varicella antigens was assessed and validated by Merck Research Laboratories (West Point, PA), using antigen-specific enzyme-linked immunosorbent assays to detect serum antibody (immunoglobulin G), before and after vaccination with MMR and varicella vaccines. Seroresponse criteria for measles, mumps, rubella, and varicella assays were predefined as ≥255 mIU/mL, ≥10 mumps antibody units per mL, ≥10 IU/mL, and ≥5 glycoprotein enzyme-linked immunosorbent assay units per mL, respectively.

Immunogenicity to influenza viruses was evaluated at MedImmune through measurement of serum hemagglutination-inhibiting (HAI) titers to each of the strains contained in the vaccine, using standard assay procedures.[12] The HAI titer was defined as the reciprocal of the highest dilution of the test serum that inhibited hemagglutination completely. A titer of <4 was assigned to serum samples for which no inhibition could be detected, even at the lowest dilution tested (1:4 dilution). A fourfold or greater difference in titer between 2 serum samples was considered significant.[13]

A secondary objective of this study was to evaluate the safety and tolerability of concurrent administration of LAIV with MMR and varicella vaccines. An adverse event (AE) was defined as any unfavorable and unintended sign, symptom, disease, or worsening of a preexisting condition associated temporally with vaccine administration. Reactogenicity events (REs) were predefined solicited AEs occurring after study vaccination, including injection site reactions and fever (>100.6°F rectal or aural, >100.0°F oral, or >99.6°F axillary). Serious AEs were defined as AEs that resulted in death, were life-threatening, required hospitalization or prolonged existing hospitalization, resulted in a persistent or significant disability, or required medical intervention to prevent one of these outcomes. To conduct the key safety comparisons between the MMR/varicella and MMR/varicella/LAIV groups (whether LAIV vaccine potentiated AEs associated with MMR/varicella vaccines), AEs and REs were recorded for 42 days after concomitant vaccination. To evaluate whether MMR/varicella vaccines potentiated REs associated with LAIV vaccine (MMR/varicella/LAIV versus LAIV alone), REs were recorded for 10 days after intranasal vaccination alone. Significant new medical conditions and serious AEs were recorded for 6 months after vaccination. REs, AEs, concomitant medication use, and health care provider visits were recorded daily by parents or guardians on assessment worksheets. Parents or guardians of children in the MMR/varicella and MMR/varicella/LAIV groups also recorded the presence and size of injection site reactions. Study staff members contacted parents or guardians by telephone to collect information regarding AEs, REs, and significant new medical conditions, ~3, 14, 28, and 42 days after concurrent administration of MMR/varicella vaccines and LAIV or placebo and 3 and 10 days after administration of LAIV or placebo alone.

Statistical Analyses

A sample size of 300 evaluable subjects per treatment group was required to provide overall power of 94% to demonstrate equivalent immune responses between the treatment groups and to provide ≥88% power to decrease the probability of an increase of >10% in the incidence of REs. The immunogenicity population included children who received all scheduled treatments, and data were analyzed according to treatment received. The safety population included children with any RE or AE data for the visit/dose-specific safety evaluation period. Data for subjects in the safety population were analyzed according to treatment received. The primary end points were to demonstrate equivalent immunogenicity of MMR vaccine and varicella vaccine after concurrent administration with intranasally administered LAIV or placebo and to demonstrate equivalent immunogenicity of intranasally administered LAIV administered alone or concurrently with MMR and varicella vaccines.

Equivalent immunogenicity of MMR and varicella vaccines with or without LAIV was evaluated by determining postvaccination seroresponse rates for measles, mumps, rubella, and varicella antigens in baseline seronegative children in the MMR/varicella/LAIV group, compared with those in the MMR/varicella group, and by comparing postvaccination geometric mean titers (GMTs) for measles, mumps, rubella, and varicella antigens in children in the MMR/varicella/LAIV group with GMTs for those in the MMR/varicella group regardless of baseline antibody titers. The definitions for seroresponse and for baseline seronegativity for measles, mumps, rubella, and varicella antigens are presented in Table 1. In the immunogenicity analysis, equivalent seroresponse was achieved if the lower limit of the 2-sided exact 95% confidence interval (CI) for the rate difference (MMR/varicella/LAIV minus MMR/varicella) was greater than −5 percentage points for measles, mumps, and rubella and greater than −10 percentage points for varicella. Equivalence based on the ratio of GMTs (MMR/varicella/LAIV GMT/MMR/varicella GMT) was achieved if the lower limit of the 2-sided 95% CI for the ratio was >0.5.

Equivalent immunogenicity of LAIV administered alone or concurrently with MMR/varicella vaccines was evaluated by determining postvaccination (dose 2) seroconversion rates for each of the vaccine strains among baseline seronegative children who received

TABLE 1 Parameters for Measles, Mumps, Rubella, Varicella, and Influenza Assays

Antigen	Units	LoD	Value Reported If Below LoD	Value Used for GMT If Below LoD	Baseline Seronegative Definition	Seroresponse Criteria
Measles	mIU/mL	120	<120	60	<255	≥255
Mumps	Mumps antibody units per mL	10	<10	5	<10	≥10
Rubella	IU/mL	10	<10	5	<10	≥10
Varicella	Glycoprotein ELISA units per mL	OD cutoff point	<0.6	0.3	<1.25	≥5
All influenza strains	Reciprocal of HAI titer	4	<4	2	≤4	≥4-fold increase from baseline

ELISA indicates enzyme-linked immunosorbent assay; LoD, limit of detection; OD, optical density.

MMR/varicella/LAIV vaccines, compared with those who received LAIV, and postvaccination (dose 2) GMTs for each of the vaccine strains among children who received MMR/varicella/LAIV vaccines, compared with those who received LAIV, regardless of baseline serostatus. The definitions for seroconversion and for baseline seronegativity for influenza strains are presented in Table 1. Equivalent seroconversion was achieved if the lower limit of the 2-sided 95% CI for the rate difference (MMR/varicella/LAIV minus LAIV) was greater than −10 percentage points for all strains. GMTs were considered equivalent if the lower limit of the 2-sided 95% CI for the ratio (MMR/varicella/LAIV GMT/LAIV GMT) was >0.5.

CIs for differences in seroresponse/seroconversion rates were constructed by using the method described by Miettinen and Nurminen.[14] CIs for all GMTs were based on the percentile-based bootstrap technique and included stratification according to season (1, 2) and continent (Australia or North America), to control for potential previous exposure to the antigens under study that might vary according to these factors.

Incidence rates of REs were analyzed with 2-sided, exact, unconditional, 90% CIs for the rate difference.[15] No formal statistical comparisons were performed for other AEs. All immunogenicity summaries and statistical analyses were performed with SAS 8 (SAS Institute, Cary, NC).

RESULTS

Study Participants

Of 1251 children assigned randomly, 1245 were evaluable for safety and immunogenicity, including 411 in the MMR/varicella group, 422 in the MMR/varicella/LAIV group, and 412 in the LAIV group. All treatment groups were well matched with regard to age, gender, and ethnicity (Table 2). Data from 1 study site (n = 6) were excluded from the analysis because the documentation of data did not meet good clinical practices standards.

A total of 1046 children (84.0%) completed the study. Subject disposition is presented in Fig 1. Ninety-eight subjects (7.9%) across the 3 treatment groups failed to meet continuing eligibility criteria and were withdrawn from the study. Of these, 49 children (50%; MMR/varicella/LAIV: 24; LAIV: 25) were withdrawn because of a local measles outbreak and subsequent unblinding of participants at 1 site; as specified in the study protocol, children who received MMR/varicella/LAIV vaccines or LAIV alone were offered open-label, measles-containing vaccine. Other reasons for failure to meet continuing eligibility criteria included history of ≥2 wheezing illnesses (n = 11), vaccine administered outside the dosing window (n = 3), varicella infection (n = 3), receipt of other vaccine (n = 7), randomization error (n = 8), egg allergy (n = 2), wheezing or bronchodilator use within 4 weeks (n = 14), and use of a product containing salicylate (n = 2).

For the evaluation of immunogenicity, 8 subjects assigned randomly to the LAIV group received the treatment regimen for the MMR/varicella/LAIV group and were summarized as MMR/varicella/LAIV subjects. The immunogenicity population therefore consisted of 411 subjects in the MMR/varicella group, 430 subjects in the MMR/varicella/LAIV group, and 404 subjects in the LAIV group.

Immunogenicity

More than 90% of evaluated subjects in the MMR/varicella/LAIV and MMR/varicella groups were seronegative for measles, mumps, rubella, and varicella antigens at baseline. Equivalent seroresponse rates were demonstrated in baseline seronegative subjects for MMR and varicella vaccines, with and without concomitant LAIV administration (Table 3). Antigen-specific GMTs for MMR and varicella with and without concurrent LAIV administration and for the MMR/varicella and MMR/varicella/LAIV groups were within the equivalence criteria. The postvaccination rubella GMT was higher in the MMR/varicella group than in the MMR/varicella/LAIV group, but both exceeded the seropositive threshold of

TABLE 2 Demographic Characteristics

	MMR/Varicella (n = 411)	MMR/Varicella/LAIV (n = 422)	LAIV (n = 412)
Age, mo			
Mean ± SD	12.8 ± 0.7	12.7 ± 0.6	12.8 ± 0.7
Median (range)	12.6 (12.0–15.9)	12.6 (12.0–15.7)	12.6 (12.0–16.0)
Male gender, n (%)	214 (52.1)	214 (50.7)	194 (47.1)
Race/ethnicity, n (%)			
White	335 (81.5)	346 (82.0)	339 (82.3)
Black	19 (4.6)	24 (5.7)	16 (3.9)
Asian/Pacific Islander	7 (1.7)	3 (0.7)	4 (1.0)
Hispanic	33 (8.0)	32 (7.6)	34 (8.3)
Other	17 (4.1)	17 (4.0)	19 (4.6)

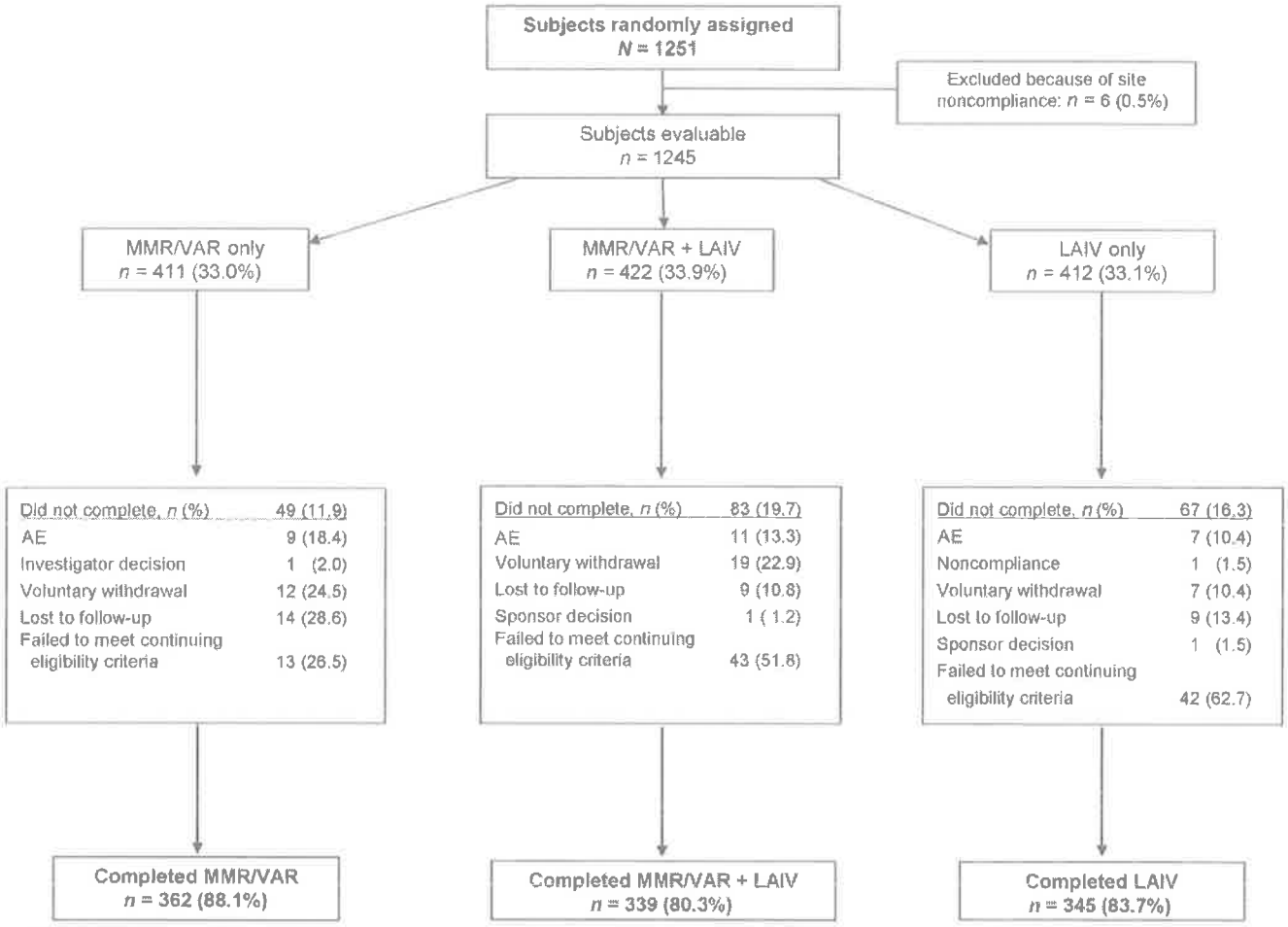

FIGURE 1
Subject disposition. Children affected by the local measles outbreak at 1 site who were withdrawn from the study are included in the "failed to meet continuing eligibility criteria" category. VAR indicates varicella.

10 IU. In contrast, the postvaccination measles titer was slightly higher in the MMR/varicella/LAIV group, compared with the MMR/varicella group (95% CI: 1.04–1.39).

Equivalent immunogenicity was also demonstrated against the 3 influenza strains contained in the vaccine (A/H1N1, A/H3N2, and B) after 2 doses of LAIV with and without concurrent administration of MMR and varicella vaccines in baseline seronegative subjects (Table 4). Strain-specific seroconversion rates (more than fourfold increase in HAI titer) in subjects who were seronegative at baseline were similar in the MMR/varicella/LAIV and LAIV groups for each of the influenza strains. Strain-specific GMTs after 2 doses of LAIV were

TABLE 3 Impact of Concurrent Administration of LAIV Vaccine on Immunogenicity of MMR and Varicella Vaccines

Antigen	Seroresponse Rates (Baseline Seronegative Subjects)[a]			GMT (All Subjects)				
	MMR/Varicella/LAIV, *n/N* (%)	MMR/Varicella, *n/N* (%)	Rate Difference (MMR/Varicella/LAIV Minus MMR/Varicella), Mean (95% CI)[b]	MMR/Varicella/LAIV		MMR/Varicella		GMT Ratio (MMR/Varicella/LAIV to MMR/Varicella), Mean (95% CI)[c]
				n	GMT	*n*	GMT	
Measles	320/330 (97.0)	329/339 (97.1)	−0.1 (−2.9 to 2.7)	344	3388.4	350	2813.6	1.21 (1.04–1.39)
Mumps	326/337 (96.7)	342/346 (98.8)	−2.1 (−4.7 to 0.1)	347	82.2	351	97.4	0.85 (0.74–0.96)
Rubella	329/338 (97.3)	340/349 (97.4)	−0.1 (−2.8 to 2.6)	344	72.6	351	102.0	0.71 (0.63–0.81)
Varicella	279/316 (88.3)	263/318 (82.7)	5.6 (0.1–11.2)	347	9.8	352	9.3	1.06 (0.95–1.18)

[a] Seronegative criteria were as follows: measles, <255 mIU/mL; mumps, <10 mumps antibody units per mL; rubella, <10 IU/mL; varicella, <1.25 glycoprotein enzyme-linked immunosorbent assay units per mL.

[b] Weighted average of the stratum-specific rate differences. Equivalence criteria were lower limit for the 95% CI of ≥5 percentage points for measles, mumps, and rubella and ≥10 percentage points for varicella; all results met the equivalence criteria.

[c] Computed with the percentile-based bootstrap technique. Equivalence criterion was lower limit for the 95% CI of >0.5; all results met the equivalence criterion.

TABLE 4 Impact of Concurrent Administration of MMR and Varicella Vaccines on Immunogenicity of 2 Doses of LAIV

Strain	Seroconversion Rates for Influenza Vaccine Strains (Baseline Seronegative Subjects)[a]			GMT for Influenza Vaccine Strains (All Subjects)				
	MMR/Varicella/LAIV, *n/N* (%)	LAIV, *n/N* (%)	Rate Difference (MMR/Varicella/LAIV Minus LAIV), Mean (95% CI)[b]	MMR/Varicella/LAIV		LAIV		GMT Ratio (MMR/Varicella/LAIV to LAIV), Mean (95% CI)[c]
				n	GMT	*n*	GMT	
A/H1N1	132/310 (42.6)	139/318 (43.7)	−1.0 (−8.7 to 6.7)	334	5.7	339	5.8	0.98 (0.85–1.13)
A/H3N2	280/286 (97.9)	294/299 (98.3)	−0.4 (−3.0 to 2.0)	334	102.9	338	112.3	0.92 (0.81–1.04)
B	305/319 (95.6)	302/328 (92.1)	3.6 (−0.2 to 7.5)	334	20.5	338	17.7	1.16 (1.03–1.30)

[a] Seronegativity was defined as baseline serum HAI titers of ≤4 for the given influenza strain.
[b] Weighted average of the stratum-specific rate differences. Equivalence criterion was lower limit for the 95% CI of ≥ −10 percentage points for all strains; all results met the equivalence criterion.
[c] Computed with the percentile-based bootstrap technique. Equivalence criterion was lower limit for the 95% CI of >0.5; all results met the equivalence criterion.

also comparable between the MMR/varicella/LAIV and LAIV groups.

Safety

All vaccine regimens were generally well tolerated. During the 42 days after the first dose of LAIV or placebo concurrent with MMR and varicella vaccines, only runny nose/nasal congestion occurred significantly more frequently among subjects who received LAIV, compared with placebo (Table 5). Nearly one half of all children in the MMR/varicella/LAIV and MMR/varicella groups experienced ≥1 AE (47% and 49%, respectively) (Table 5). The most frequently reported AEs during the 42 days after concurrent vaccination with MMR/varicella/LAIV vaccines or MMR/varicella vaccines were diarrhea (17% vs 15%) and otitis media (8% vs 11%). Respiratory AEs occurred less frequently in the MMR/varicella/LAIV group than in the MMR/varicella group, with more than twice as many children in the MMR/varicella group reporting wheezing (2.5%), compared with the MMR/varicella/LAIV group (1.2%).

Irritability and fever were significantly more frequent within 10 days after vaccination in subjects who received LAIV concurrent with MMR and varicella vaccines than in subjects who received LAIV alone (Table 6). There were no significant differences in REs within 10 days after the second dose of LAIV whether the first dose was administered concurrent with MMR and varicella vaccines or alone; ≥1 AE was reported by 25% and 32% of children, respectively, within 10 days after vaccination (Table 6). Respiratory events occurred less frequently in the MMR/varicella/LAIV group than in the LAIV group (1.5% vs 4.1%, respectively). No AE was reported with a frequency of >10%.

No deaths were reported during the study. Nine reported serious AEs were considered to be possibly related to study vaccine. In the MMR/varicella group, there were 2 cases of croup, 1 case of pneumonia, and 1 case of bronchiolitis. In the MMR/varicella/LAIV group, there was 1 case each of croup and bronchiolitis. In the LAIV group, there was 1 case each of a viral chest infection, bronchiolitis, and bronchospasm. Nine children experienced 9 significant new medical conditions, including asthma (1 in the MMR/varicella group and 3 in the LAIV group), speech delay (2 in the LAIV group), excessive language delay (1 in the MMR/varicella group), cerebral palsy (1 in the MMR/varicella/LAIV group), and seizures (1 in the LAIV group).

DISCUSSION

Influenza is associated with a significant excess of outpatient visits, hospitalizations, and rare deaths among young children each year.[16–20] Routine annual influenza vaccination is now recommended for children between the ages of 6 months and 59 months, adding multiple vaccinations to the pediatric immunization schedule.[21]

The Advisory Committee on Immunization Practices recommends that injectable or nasally administered live vaccines not administered on the same day should be administered >4 weeks apart whenever possible, to minimize the potential for interference.[22] This recommendation is supported by the observation that immunization with a live measles vaccine can block the immune responses to a live smallpox vaccine if the measles vaccine is administered within 15 days after smallpox vaccine dosing but not if the vaccines are administered simultaneously, presumably because of the action of interferon induced in response to the initial live virus vaccine.[23,24] Similarly, administration of varicella vaccine within 28 to 30 days after receipt of MMR vaccine, but not simultaneous administration, has been associated with an increased risk of breakthrough varicella disease.[25,26] However, concomitant administration of live vaccines can produce interference; concomitant administration of 2 live oral vaccines (polio vaccine and rotavirus vaccine) has been associated with a >40% reduction in seroresponse rates to a live oral rotavirus vaccine.[27,28] Because it is common practice for children to receive several vaccines during the same office or clinic visit, it is important to establish the safety and immunogenicity of concomitantly administered live virus vaccines.

To date, limited data have been published on the impact on other vaccines of concurrent administration of either inactivated or live influenza virus vaccines, with respect to the immune responses of children or adults. The findings of the current study indicate equivalent immunogenicity with concurrent administration of MMR and varicella vaccines with LAIV, compared with separate administration. Seroresponse rates and ratios of antigen-specific antibody titers for measles, mumps, rubella, and varicella antigens present in the vaccines were

TABLE 5 REs and AEs Reported Within 42 Days After Dose 1 of MMR/Varicella/LAIV or MMR/Varicella Vaccines

	n (%)	
	MMR/Varicella/LAIV	MMR/Varicella
REs		
Evaluable	412 (95.8)	393 (95.6)
Any RE	389 (94.4)	366 (93.1)
Cough	211 (51.2)	204 (51.9)
Runny nose/nasal congestion	346 (84.0)[a]	305 (77.6)[a]
Sore throat	62 (15.0)	50 (12.7)
Irritability	296 (71.8)	276 (70.2)
Headache	18 (4.4)	15 (3.8)
Chills	15 (3.6)	8 (2.0)
Vomiting	97 (23.5)	89 (22.6)
Muscle aches	11 (2.7)	9 (2.3)
Decreased activity	113 (27.4)	97 (24.7)
Fever		
>100°F oral or equivalent	270 (65.5)	238 (60.6)
>102°F oral or equivalent	96 (23.3)	83 (21.1)
Any rash/injection site reaction	193 (46.8)	196 (49.9)
Varicella-like rash	16 (3.9)	21 (5.3)
Measles-like rash	23 (5.6)	18 (4.6)
Other/unknown rash	99 (24.0)	94 (23.9)
AEs[b]		
Evaluable	410 (95.3)	394 (95.9)
Any AE	191 (46.6)	191 (48.5)
Body as a whole		
Infection	6 (1.5)	6 (1.5)
Fungal infection	6 (1.5)	5 (1.3)
Viral infection	7 (1.7)	10 (2.5)
Injection site bruise	5 (1.2)	1 (0.3)
Accidental injury	23 (5.6)	19 (4.8)
Digestive system		
Anorexia	13 (3.2)	15 (3.8)
Diarrhea	70 (17.1)	59 (15.0)
Gastroenteritis	6 (1.5)	4 (1.0)
Nervous system, sleep disorder	9 (2.2)	5 (1.3)
Respiratory system		
Bronchiolitis	0 (0.0)	4 (1.0)
Bronchitis	0 (0.0)	7 (1.8)
Croup	6 (1.5)	7 (1.8)
Epistaxis	2 (0.5)	4 (1.0)
Pharyngitis	8 (2.0)	12 (3.0)
Sinusitis	5 (1.2)	4 (1.0)
Sneezing	8 (2.0)	8 (2.0)
Wheezing	5 (1.2)	10 (2.5)
Skin		
Eczema	4 (1.0)	6 (1.5)
Rash	8 (2.0)	8 (2.0)
Special senses		
Conjunctivitis	12 (2.9)	13 (3.3)
Ear infection, undifferentiated	7 (1.7)	3 (0.8)
Otitis media	33 (8.0)	43 (10.9)
Pain ear	4 (1.0)	3 (0.8)

[a] Difference between treatment groups was significant, on the basis of the 2-sided 95% CI of the difference (MMR/varicella/LAIV minus MMR/varicella).

[b] AEs reported for ≥1% of children.

TABLE 6 REs and AEs Reported Within 10 Days After Dose 1 of MMR/Varicella/LAIV or LAIV Vaccines

	n (%)	
	MMR/Varicella/LAIV	LAIV
REs		
Evaluable	412 (95.8)	388 (96.0)
Any RE	364 (88.3)	325 (83.8)
Cough	119 (28.9)	118 (30.4)
Runny nose/nasal congestion	293 (71.1)	271 (69.8)
Sore throat	19 (4.6)	24 (6.2)
Irritability	249 (60.4)[a]	200 (51.5)[a]
Headache	12 (2.9)	4 (1.0)
Chills	5 (1.2)	7 (1.8)
Vomiting	59 (14.3)	36 (9.3)
Muscle aches	7 (1.7)	3 (0.8)
Decreased activity	72 (17.5)	57 (14.7)
Fever		
>100°F oral or equivalent	213 (51.7)[a]	113 (29.1)[a]
>101°F oral or equivalent	121 (29.4)[a]	54 (13.9)[a]
>102°F oral or equivalent	67 (16.3)[a]	30 (7.7)[a]
>103°F oral or equivalent	13 (3.2)	9 (2.3)
>104°F oral or equivalent	3 (0.7)	5 (1.3)
AEs[b]		
Evaluable	412 (95.8)	388 (96.0)
Any AE	103 (25.0)	125 (32.2)
Body as a whole		
Injection site bruise	5 (1.2)	0 (0.0)
Accidental injury	6 (1.5)	9 (2.3)
Digestive system		
Anorexia	9 (2.2)	13 (3.4)
Diarrhea	39 (9.5)	33 (8.5)
Nervous system		
Insomnia	0 (0.0)	4 (1.0)
Sleep disorder	7 (1.7)	5 (1.3)
Respiratory system		
Epistaxis	1 (0.2)	5 (1.3)
Sneezing	4 (1.0)	6 (1.5)
Wheezing	1 (0.2)	5 (1.3)
Skin, rash	4 (1.0)[c]	27 (7.0)
Special senses		
Conjunctivitis	6 (1.5)	11 (2.8)
Ear infection, undifferentiated	4 (1.0)	2 (0.5)
Otitis media	8 (1.9)	11 (2.8)

[a] Difference between treatment groups was significant, on the basis of 2-sided 95% CIs of the difference (MMR/varicella/LAIV minus LAIV).

[b] AEs reported for ≥1% of children.

[c] Rash was intended to be collected as a RE for the MMR/varicella/LAIV group (see Table 5), although in some cases rash was reported as an AE. For the MMR/varicella/LAIV group, the incidence of "other/unknown rash" collected as a RE within 10 days after dose 1 was 11.9%.

similar, regardless of whether MMR and varicella vaccines were administered concurrently with LAIV or concurrently with placebo. Similarly, responses elicited by 2 intranasal doses of LAIV were not affected by concomitant administration of subcutaneously administered MMR and varicella vaccines. The serum HAI responses to the 3 LAIV strains observed in this study are consistent with findings from other LAIV studies, including the lower immune response to the A/H1N1 strain.[3,29,30] Overall, although the presence of antibody responses after the administration of LAIV is predictive of protection, the lack of an antibody response is not indicative of the absence of protection.[31]

REs reported after vaccinations in this study were generally typical of those observed in a young pediatric population after vaccination. The increased incidence of fever seen in children treated with MMR/varicella/LAIV vaccines, compared with those treated with LAIV alone, can

be attributed in large part to receipt of MMR and varicella vaccines, because an increased incidence of fever was reported in previous studies of concurrent immunization with MMR and varicella vaccines.[32,33] Of note, respiratory AEs (including wheeze) were less frequent in the MMR/varicella/LAIV group than in the MMR/varicella group in the 42 days after vaccination.

CONCLUSIONS

Concomitant administration of live vaccines (MMR, varicella, and LAIV vaccines) to children 12 to 15 months of age did not affect significantly the seroresponse rates for MMR and varicella vaccines with simultaneous administration of LAIV. Strain-specific seroconversion rates for each of the 3 LAIV vaccine strains were not altered by concomitant administration of MMR and varicella vaccines. Concurrent administration of MMR vaccine, varicella vaccine, and intranasally administered LAIV was generally well tolerated. These findings suggest that LAIV can be administered concomitantly to young children with MMR and varicella vaccines in routine clinical practice with no diminution of immunogenicity or safety. This is important because LAIV offers potential benefits to young children, such as a broad immune response that includes both systemic and mucosal antibody responses and cellular immune responses,[34] protection against strains that are antigenically "drifted" from the vaccine strains,[4,35–38] and needle-free, intranasal administration.

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MAJOR ARTICLE

Phase 2 Evaluation of Parainfluenza Type 3 Cold Passage Mutant 45 Live Attenuated Vaccine in Healthy Children 6–18 Months Old

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A phase 2 evaluation of live attenuated parainfluenza type 3 (PIV3)–cold passage mutant 45 (cp45) vaccine was conducted in 380 children 6–18 months old; 226 children (59%) were seronegative for PIV3. Of the 226 seronegative children, 114 received PIV3-cp45 vaccine, and 112 received placebo. No significant difference in the occurrence of adverse events (i.e., runny nose, cough, or temperature ≥38°C) was noted during the 14 days after vaccination. There was no difference between groups in the occurrence of acute otitis media or serous otitis media. Paired serum samples were available for 109 of the seronegative vaccine recipients and for 110 of the seronegative placebo recipients; 84% of seronegative vaccine recipients developed a ≥4-fold increase in antibody titers. The geometric mean antibody titer after vaccination was 1:25 in the vaccine group and <1:4 in the placebo group. PIV3-cp45 vaccine was safe and immunogenic in seronegative children and should be evaluated for efficacy in a phase 3 field trial.

Human parainfluenza viruses (PIVs) are important causes of serious respiratory tract disease in infants and young children. According to the US Institute of Medicine, 25% of children <5 years old experience a clinically significant PIV infection annually, and ~2% of PIV-infected infants will require hospitalization [1]. Four types of PIV are associated with respiratory illness in young infants and children. Of special significance

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is PIV type 3 (PIV3), which causes pneumonia and bronchiolitis and ranks second only to respiratory syncytial virus (RSV) as a cause of bronchiolitis and pneumonia in infants <6 months old [2–4]. PIV3 can cause severe disease throughout the first 2 years of life, and virtually all children have experienced primary PIV3 infections by age 3–4 years. Overall, PIV3 is considered to be responsible for ~11% of hospitalizations for pediatric respiratory tract disease in the United States [1].

Past attempts to develop inactivated PIV3 vaccines showed that resistance to disease was not induced, despite the development of serum antibodies after vaccination [5]. Protection against PIV3 in humans is most likely to be achieved by the induction of both circulating and mucosal antibodies that are active against the hemagglutinin-neuraminidase (HN) glycoprotein (the attachment protein) and the fusion glycoprotein, 2 surface antigens that induce protective neutralizing antibodies [6, 7]. Such protective mucosal and circulating antibodies should be induced most efficiently by delivery of a live attenuated virus vaccine to the mucosa of the respiratory tract [8].

The live attenuated virus vaccine evaluated in the present study, JS strain, was derived from human wild-type PIV3 that was originally isolated from a 1-year-old child with a febrile respiratory illness [9–11]. Several attenuated mutants of the JS strain were derived by passaging the virus numerous times in primary monkey kidney cells at sequentially lower temperatures that are suboptimal for PIV3 replication. After 12, 18, and 45 cold passages, mutants cp12, cp18, and cp45, respectively, were isolated and characterized. During the process of low-temperature passage, each of the mutants acquired 3 phenotypic markers: cold adaptation (ca; the ability to replicate efficiently in vitro at the suboptimal temperature of 20°C), temperature sensitivity (ts; restricted growth at 39°C in tissue culture), and attenuation, manifested by restricted replication in hamsters and chimpanzees, compared with that of wild-type virus [9–12].

Phase 1 studies of PIV3-cp45 vaccine, which was produced in fetal rhesus monkey lung (FRhL-2) tissue culture cells, have been conducted in seropositive children 6 months to 10 years old, seronegative infants 6–36 months old, and infants 1–2 months old [13, 14]. In general, the vaccine appeared to be satisfactorily attenuated, infectious, immunogenic, and phenotypically stable. However, because PIV3-cp45 replicates to modest titers on monolayer cultures of FRhL-2 cells, production of vaccine virus in this cell line would be inefficient for larger-scale manufacturing; thus, an easily scalable and cost-effective production process for PIV3 that propagates the virus in Vero cells grown on microcarrier beads in a bioreactor was developed by Wyeth Vaccines Research (Pearl River, NY).

In phase 1 studies, PIV3-cp45 grown in Vero tissue culture was generally well tolerated by all cohorts, with the exception that, in the seronegative cohort, otitis media (OM) was observed in 3 of 32 vaccine recipients and in none of 14 placebo recipients [15]. Interpretation of the significance of this finding was uncertain because of the frequent acquisition of other intercurrent viral infections. The overall rates of upper respiratory–tract illness (URI) were very similar in the vaccine and placebo groups, and PIV3 isolates recovered from these children retained the ts phenotype [15]. Thus, as with PIV3-cp45 grown in FRhL, the vaccine virus grown in Vero tissue culture appeared to be satisfactorily attenuated, infectious, immunogenic, and phenotypically stable. We therefore undertook a phase 2 study of PIV3-cp45 vaccine, to compare the safety profile of a dose of 10^5 pfu administered intranasally (inl) to children 6–18 months old with that of placebo and to assess the immunogenicity of the vaccine. The phase 2 study was sufficiently powered to evaluate the frequency of OM in vaccine and placebo groups.

SUBJECTS, MATERIALS, AND METHODS

Study design and vaccine. We enrolled ~400 healthy subjects 6–18 months old in a multicenter, double-blind, placebo-controlled safety and immunogenicity trial. This sample size was chosen to enroll ~200 PIV-seronegative children. Eligible subjects were assigned to receive the investigational study vaccine according to a randomization schedule generated by the sponsor's statistician and provided in sealed randomization envelopes to study personnel at each site responsible for preparing study vaccine for administration. Each subject was randomized to receive either a single dose of PIV3-cp45 at 1×10^5 pfu or placebo (PBS with sucrose, phosphate, and glutamate) inl as nose drops instilled while the subject was supine. Randomization was a 1:1 ratio of vaccine to placebo.

Subjects. Study subjects were healthy children 6–18 months old, whose parents or guardians gave written, informed consent. The human-experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions were followed in the conduct of this clinical research. Each subject's history was reviewed, and a physical examination was performed to verify that the health and development of all subjects were normal. Subjects with any of the following conditions or characteristics were excluded from study enrollment or from continued participation: immunosuppression or taking immunosuppressive medication; serious chronic illness; cardiac or respiratory illness, including those with >1 prior episode of wheezing (including illnesses diagnosed as asthma or reactive airway disease) confirmed by a physician or subjects with pressure equalization tubes; members of a household with a pregnant woman, an immunocompromised individual, or an infant <6 months old; or attendance at day care with infants <6 months old. Attendance at a day-care facility in which children were separated by age was acceptable if the vaccine recipient did not spend any time in the area designated for infants <6 months old and if conditions

pertaining to any common area of the facility minimized opportunities for transmission of virus through direct physical contact between children or by the aerosol route. Subjects with self-limiting illnesses were included after the condition resolved and if no other exclusion criteria were met. These exclusion criteria included acute febrile illness (≥38°C), acute OM (AOM), receipt of short-term antibiotic therapy for acute illness, receipt of any vaccine within the previous 2 weeks, receipt of any live vaccine within the previous 4 weeks, or receipt of gamma globulin within the past 3 months. Infants born at <37 weeks of gestation were deferred from study participation until they were at least 1 year old. Children did not receive other vaccines for 42 days after enrollment.

Procedures. Serum samples were obtained before inl vaccination, to determine prevaccination antibody levels. Subjects received either the vaccine or the placebo by nose drops in a volume of 0.25 mL/nostril, for a total dose of 0.5 mL. Vaccinations were administered between 28 October 1998 and 13 November 2000, and vaccinations were not given during winter, to reduce intercurrent wild-type viral infection. The parents were asked to keep track of any illness or symptoms on a parent diary card each day for 14 days after vaccination; electronic thermometers were provided, and parents were asked to obtain daily oral, rectal, or axillary temperatures. Parents were asked to record the child's temperature at bedtime daily for 14 days after vaccination and whenever the child felt warm during the 42 days after vaccination. Children were seen by study personnel twice in the 2 weeks after vaccination (day 7 and 14, ±1 day). These brief visits (20–30 min each) allowed the study staff to examine the child closely for any signs of a runny nose, sore throat, fever (temperature ≥38°C), cough, respiratory illness, or an ear infection. Study staff also contacted the parents by telephone on days 21, 28, and 35 (±2 days), to inquire if the child had experienced any symptoms of illness. Six weeks after the first vaccination visit, all enrolled children returned to the clinic for a brief physical examination, and a blood sample was obtained to measure the child's antibody response to the vaccination.

During the 42 days of the study, a clinician was available 24 h/day to examine ill children. An examination was performed if a child had a rectal temperature ≥38°C (or equivalent if oral or axillary temperatures were taken), respiratory illness, or symptoms suggestive of an ear infection. For all study subjects, fever, URI (rhinorrhea or pharyngitis), cough, and lower respiratory–tract illness (LRI) were defined as described elsewhere [14]. During each illness, the child's ears were examined for signs of an ear infection, and a nasal-wash sample was obtained for viral culture to determine whether there was an intercurrent viral infection. Otoscopy was performed at each clinic visit at which the child was well. AOM was defined as an inflamed, immobile tympanic membrane, with or without bulging, observed by a physician or nurse practitioner and confirmed by tympanometry or a second observer. These findings were noted independently of fever or other respiratory symptoms. An abnormal tympanogram alone was not considered to be diagnostic of AOM. Serous OM was defined as all other cases of OM not fitting the above strict criteria. All children were examined at the end of the study: day 42 (range, 35–66 days) after vaccination. Serum samples from all subjects were tested for antibodies to PIV3 (Washington/57 strain) by the hemagglutination inhibition (HAI) antibody test [13], starting at a serum dilution of 1:4; children with a titer of ≤1:8 were considered to be seronegative. Vaccine virus shedding was not routinely determined in the present study, to avoid any confounding clinical findings that may be caused by the frequent nasal-wash samples that are necessary to obtain samples for viral cultures.

Statistics. The event of primary analysis was AOM in seronegative subjects. It was estimated that ~50% of the enrolled subjects would be seronegative. This sample size was sufficient to reject the hypothesis, with a power of 82%, that the rate of OM in the vaccine group was ≥11% higher (90% confidence interval, upper bound) than the rate of OM in the placebo group, assuming that the placebo rate was 10%. For analysis of possible adverse reactions to vaccination (i.e., runny nose, cough, or fever) Fisher's exact test *P* values were adjusted for each symptom or sign or each day by Bonferroni's method. Fisher's exact test was used to compare the occurrence of OM in vaccine recipients and placebo recipients.

RESULTS

Enrollment. Three hundred eighty children were enrolled in the study and were given either PIV3-cp45 vaccine or placebo inl. Of those 380 children, 226 (59%) were seronegative (antibody to PIV3 ≤1:8 by HAI antibody test). Of the 226 seronegative children, 114 received PIV3-cp45 vaccine, and 112 received placebo. Three hundred seventy-two (97.9%) completed the 42 days of study.

Signs and symptoms of respiratory disease. Figure 1*A*, 1*B*, and 1*C* illustrates, in the seronegative cohort, the daily frequency of runny nose, cough, or fever during the 14 days in which parents recorded symptoms on diary cards. There was no statistically significant difference between groups in the frequency of runny nose, cough, or fever on any day. In both the vaccine and placebo groups, children selected on day 0 at time 0 for the absence of runny nose, cough, or fever experienced an increase in these events during the first days of the study. This is most dramatically seen for the occurrence of runny nose (figure 1*A*). Children with runny nose were excluded at entry, and, therefore, the occurrence of runny nose increased during the study in both the placebo and the vaccine recipients; this

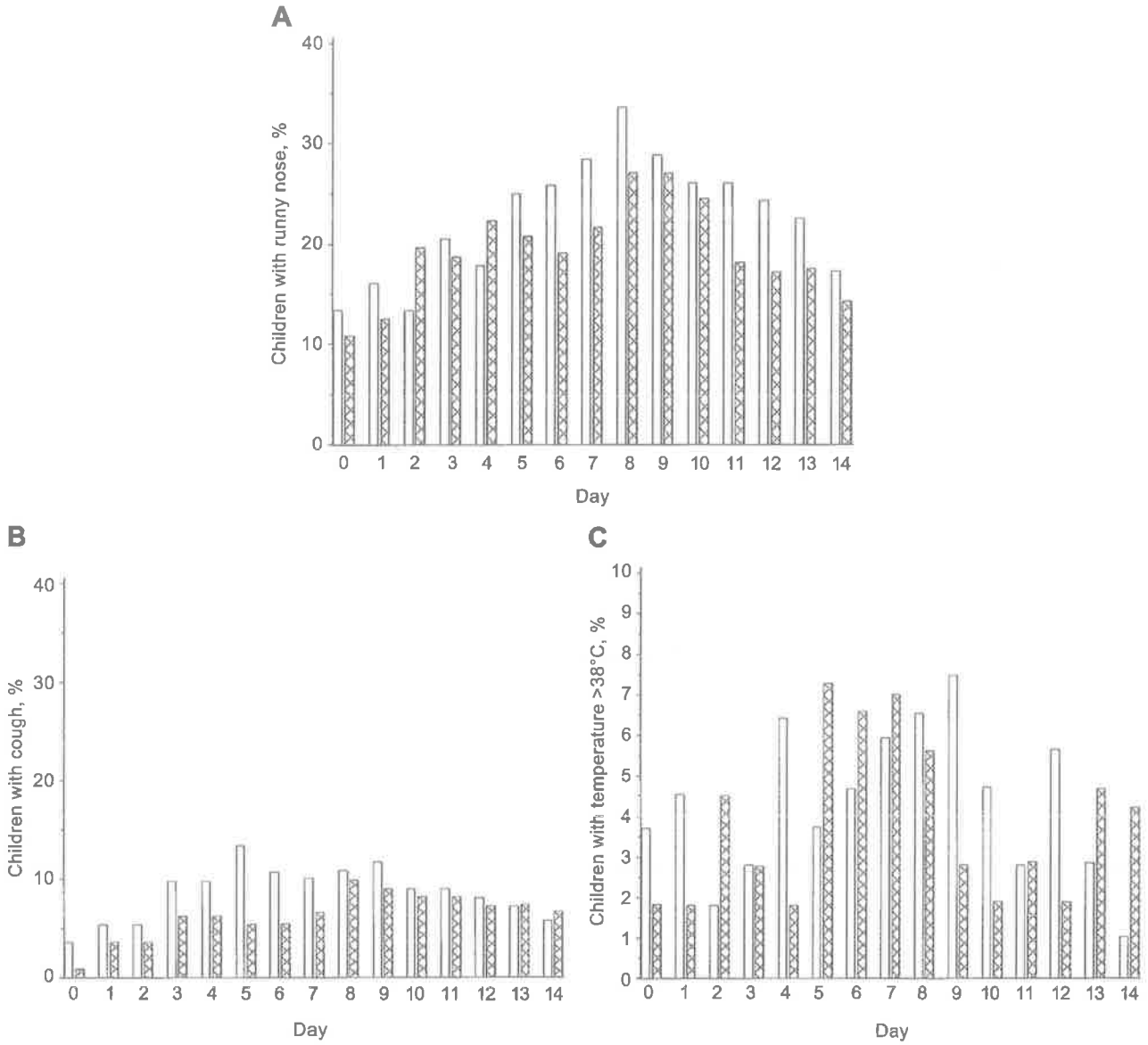

Figure 1. *A,* Percentage of parainfluenza virus 3 (PIV3)–seronegative children with runny nose on days 0–14 after intranasal (inl) vaccination with PIV3–cold passage mutant 45 (cp45) *(hatched bars)* or placebo *(white bars).* No statistically significant differences between vaccine and placebo groups occurred on any day ($P > .05$, Fisher's exact test with Bonferroni's correction). *B,* Percentage of PIV3-seronegative children with cough on days 0–14 after inl vaccination with PIV3-cp45 *(hatched bars)* or placebo *(white bars).* No statistically significant differences between vaccine and placebo groups occurred on any day ($P > .05$, Fisher's exact test with Bonferroni's correction). *C,* Percentage of PIV3-seronegative children with fever (temperature ≥38°C) on days 0–14 after inl vaccination with PIV3-cp45 *(hatched bars)* or placebo *(white bars).* No statistically significant differences between vaccine and placebo groups occurred on any day ($P > .05$, Fisher's exact test with Bonferroni's correction).

illustrates the phenomenon of return to the mean, whereby, on any given day, ~20% of young children have runny nose, as seen in other similar studies [16]. A similar phenomenon was observed for cough and fever, with baseline occurrence of cough in ~10% of the children after day 7, and the baseline occurrence of fever in ~3%–6% of children, depending on the day observed. Among the seropositive children, the frequency of signs and symptoms of respiratory illness (including runny nose, cough, or fever) was not significantly different on any day (data not shown). There was no difference in the frequency of children with findings of LRI (9/189 in the vaccine group vs. 12/191 in the placebo group; $P = .65$, Fisher's exact test).

Isolation of viruses during illness episodes. In the present study, nasal-wash samples were obtained only during illness visits. A total of 19 vaccine virus isolates were recovered from the nasal-wash samples of 17 (9.0%) of 189 vaccine recipients during these unscheduled visits. The majority of the isolates (17/19 [89.5%]) were recovered from seronegative subjects (ta-

Table 1. Isolation of vaccine and wild-type parainfluenza virus 3 (PIV3) from subjects, by prevaccination serostatus and study group.

Study interval, isolate phenotype	Seronegative subjects		All subjects	
	PIV3-cp45 (n = 114)	Placebo (n = 112)	PIV3-cp45 (n = 189)	Placebo (n = 191)
Days 1–11				
Vaccinelike[a]	15	0	17[b]	0
Wild type	0	2	1[b]	2
Days 12–22				
Vaccinelike	1	0	1	0
Wild type	1	1	1	1
Days ⩾23				
Vaccinelike	0	0	0	0
Wild type	2	4	2	5
Any day				
Vaccinelike	16	0	17[b]	0
Wild type	3	7	4[b]	8

NOTE. Data are no. of subjects shedding the indicated virus. cp45, Cold passage mutant 45.

[a] Temperature-sensitive and cold-adapted phenotypes.

[b] Three subjects who shed PIV3 (subject 1024 shed vaccine virus, subject 1829 shed wild-type virus, and subject 1828 shed both strains) were placed in the "all" category because their pretreatment blood samples were either unavailable (1829) or were obtained too many days before vaccination to assure a seronegative status at time of vaccination.

ble 1). All vaccine isolates were recovered within the first 14 days after vaccination. Both vaccine and wild-type PIV3 was detected in the nasal-wash sample of 1 subject during an illness visit. Three subjects who shed PIV3 (1 vaccine, 1 wild type, and 1 both strains) were placed in the "all" category because their pretreatment blood sample was either unavailable (n = 1) or was collected too long before vaccination (n = 2) to assure a seronegative status at time of vaccination.

To determine whether the higher proportion of vaccine isolates recovered from seronegative subjects was due to this cohort's lack of preexisting antibody to PIV3 or to more-frequent sampling of these subjects, nasal-wash sample collection rates among seronegative and seropositive subjects were compared and were found to be statistically similar ($P > .90$). Overall, 137 (60.6%) of 226 seronegative subjects and 95 (61.7%) of 154 seropositive subjects enrolled in the study had at least 1 nasal-wash sample obtained after vaccination. Within the first 11 days after vaccination, the nasal-wash sample collection rates were also similar between cohorts, with 71 (31.4%) of 226 seronegative subjects and 42 (27.3%) of 154 seropositive subjects having at least 1 nasal-wash sample obtained. Wild-type PIV3 was isolated from 4 (2.1%) of 189 vaccine recipients and 8 (4.2%) of 191 placebo recipients in the study (P = .38, Fisher's exact test).

Children enrolled in the present study experienced intercurrent infections with respiratory pathogens other than PIV3. PIV1, PIV2, RSV, adenovirus, cytomegalovirus, enterovirus, and rhinovirus were isolated from subjects enrolled in the present study. With the exception of PIV3, the most commonly recovered pathogens were RSV (2.6% of vaccine recipients and 1.6% of placebo recipients), adenovirus (1.1% of vaccine recipients and 3.7% of placebo recipients), enterovirus (3.2% of vaccine recipients and 2.1% of placebo recipients), and rhinovirus (1.1% of vaccine recipients and 2.1% of placebo recipients). In general, these viruses were isolated from children in both treatment groups throughout the entire study period, with no temporal relationship to vaccination. Among the 9 (4.8%) vaccine recipients and 12 (6.3%) placebo recipients who had evidence of LRI, viruses were recovered from 3 vaccine recipients (1 isolate each of PIV2, RSV, and vaccine virus) and from 5 placebo recipients (RSV, PIV3 [wild type, in 2 subjects], PIV1, and rhinovirus).

AOM. The occurrence of AOM among the seronegative subjects and among all subjects is summarized in table 2. The occurrence of AOM was common in both the vaccine recipients and placebo recipients and was divided into that occurring during the early postvaccination interval (days 1–11, the period of peak vaccine virus replication [13–15]), the interval when most vaccine virus replication had waned to absent or low levels (days 12–22), and the late postvaccination period, when the

Table 2. Subjects experiencing acute otitis media in the seronegative cohort and in all subjects, regardless of antibody status.

Cohort, days	Group		Vaccine group % − placebo group %	90% CI[a]
	PIV3-cp45	Placebo		
Seronegative[b]				
1–11	5 (4.4)	7 (6.3)	−1.9	−9.6 to 5.5
12–22	9 (7.9)	8 (7.1)	0.8	−6.8 to 9.6
23–42	10 (8.8)	8 (7.1)	1.6	−6.0 to 10.7
1–42	20 (17.5)	17 (15.2)	2.4	−6.9 to 13.0
Any	21 (18.4)	18 (16.1)	2.3	−7.1 to 13.1
All[c]				
1–11	10 (5.3)	10 (5.2)	0.1	−5.2 to 5.4
12–22	16 (8.5)	13 (6.8)	1.7	−4.2 to 7.7
23–42	20 (10.6)	12 (6.3)	4.3	−1.7 to 10.5
1–42	38 (20.1)	26 (13.6)	6.5	−0.8 to 14.0
Any	39 (20.6)	27 (14.1)	6.5	−0.9 to 14.1

NOTE. Data are no. (%) of subjects, except where noted. PIV3-cp45, parainfluenza virus 3–cold passage mutant 45.

[a] The 2-sided confidence intervals (CIs) were calculated by use of StatXact (Cytel). 90% CIs represent the 90% CI for the percentage of subjects in the PIV3-cp45 vaccine group with acute otitis media minus the percentage of subjects in the placebo group and, in each case, includes 0%. The denominator is the no. of randomized subjects.

[b] In the seronegative cohort, 114 subjects received PIV3-cp45, and 112 subjects received placebo.

[c] In the "all" cohort, 189 subjects received PIV3-cp45, and 191 subjects received placebo.

viral cultures were generally negative for vaccine virus (days 23–42). There was no statistically significant difference between groups in the frequency of AOM at any of these intervals nor did the overall total number of AOM cases differ between groups. The occurrence of all OM, defined as any evidence of AOM or serous OM, is shown in table 3. There was no significant difference between groups or among all subjects in the occurrence of all OM, regardless of serostatus.

Immunogenicity. Paired serum samples were available for 109 of the seronegative vaccine recipients and for 110 of the seronegative placebo recipients. The antibody response to PIV3 is summarized in figure 2 and table 4. Eighty-six of the seronegative vaccine recipients (79%) developed ≥4-fold increase in antibody titer or seroconversion from ≤1:8 to ≥1:16. After vaccination, the geometric mean antibody titer was 1:25 among the vaccine recipients. In contrast, only 13 (12%) of 110 placebo recipients had an antibody response after vaccination. These 13 children likely had intercurrent natural infection with wild-type PIV3, which was circulating in the community at the time of the study. Postvaccination geometric mean titer was <1:4 in the placebo recipients. Among the seropositive subjects, prevaccine antibody titer was 1:50 and did not increase after vaccination (figure 2*B* and table 4).

Table 3. Subjects experiencing either acute otitis media or serous otitis media in the seronegative cohort and in all subjects, regardless of antibody status.

Cohort, days	Group		Vaccine group % − placebo group %	90% CI[a]
	PIV3-cp45	Placebo		
Seronegative[b]				
1–11	11 (9.6)	12 (10.7)	−1.1	−10.1 to 7.6
12–22	18 (15.8)	20 (17.9)	−2.1	−12.2 to 7.8
23–42	15 (13.2)	15 (13.4)	−0.2	−9.8 to 9.1
1–42	34 (29.8)	33 (29.5)	0.4	−10.1 to 12.2
Any	35 (30.7)	34 (30.4)	0.3	−10.2 to 12.2
All[c]				
1–11	19 (10.1)	20 (10.5)	−0.4	−7.3 to 5.6
12–22	28 (14.8)	32 (16.8)	−1.9	−9.7 to 4.9
23–42	28 (14.8)	27 (14.1)	0.7	−6.4 to 7.9
1–42	56 (29.6)	53 (27.7)	1.9	−6.4 to 10.3
Any	57 (30.2)	54 (28.3)	1.9	−6.5 to 10.4

NOTE. Data are no. (%) of subjects, except where noted. PIV3-cp45, parainfluenza virus 3–cold passage mutant 45.

[a] The 2-sided confidence intervals (CIs) were calculated by use of percentage of subjects in the placebo group and, in each case, includes 0%. The denominator is the no. of randomized subjects.

[b] In the seronegative cohort, 114 subjects received PIV3-cp45, and 112 subjects received placebo.

[c] In the "all" cohort, 189 subjects received PIV3-cp45, and 191 subjects received placebo.

DISCUSSION

Previous clinical trials have confirmed the viral-shedding pattern, genetic stability, and immunogenicity of Vero tissue culture–produced PIV3-cp45 vaccine in adults, children, and infants [15]. A single dose of 10^6 pfu of PIV3-cp45 was evaluated in adults and seropositive children, single doses of 10^4 or 10^5 pfu were evaluated in seronegative children, and 2 doses at 1- or 3-month intervals were evaluated in infants 1–2 months old [15]. Ninety-four percent of seronegative vaccinated children and 94% of vaccinated infants were infected by the vaccine virus after 1 dose. Signs and symptoms of mild respiratory illness were common in both vaccine and placebo groups of seronegative children and infants and occurred in up to one-half of placebo recipients; OM was reported in 3 of 32 vaccinated children. These and other similar phase 1 studies have been effective screening studies to eliminate insufficiently attenuated or overly attenuated live attenuated vaccine candidates, but phase 1 studies are limited in assessing with precision the possible association of OM with vaccine because of study size [15, 17]. The present phase 2 evaluation was specifically undertaken to assess the frequency of common signs and symptoms of AOM and to obtain more-precise estimates of the frequency of signs and symptoms of URI that might be caused by vaccine virus replication.

The occurrence of runny nose and fever increased in both the vaccine and placebo groups during the 7 days after vaccination. Children who were selected for enrollment were afebrile and did not exhibit runny nose or cough. The diary card information recorded by parents noted that, later on the day of vaccination, 11% of children who received vaccine and 13% of children who received placebo exhibited runny nose. Cough and fever were also present in some children on the day of vaccination, but not more frequently in either group. On subsequent days, the frequency of runny nose increased and peaked on day 8 (34% in the placebo group and 28% in the vaccine group) before returning to a level of 16%–18% on day 14, after which data were not collected. We believe that this increase in the frequency of runny nose is a result of the selection of children on day 0 without manifestations of URI and that a return to the mean baseline values of ~20% of children with runny nose accounts for much of this observation. Selection of well children without symptoms of URI may result in the selection of children who are susceptible to prevalent viruses causing URI in the community, and the peak incidence of symptoms of URI on day 8 may represent acquisition of these agents by both the vaccine and placebo groups. In contrast to live attenuated influenza vaccine, which is associated with a slight increase in fever on day 2 after vaccination and runny nose on days 2, 3, 7, and 8 after vaccination [16], an increase in these minor events was not observed in the present study of PIV3-cp45; these findings further indicate the highly atten-

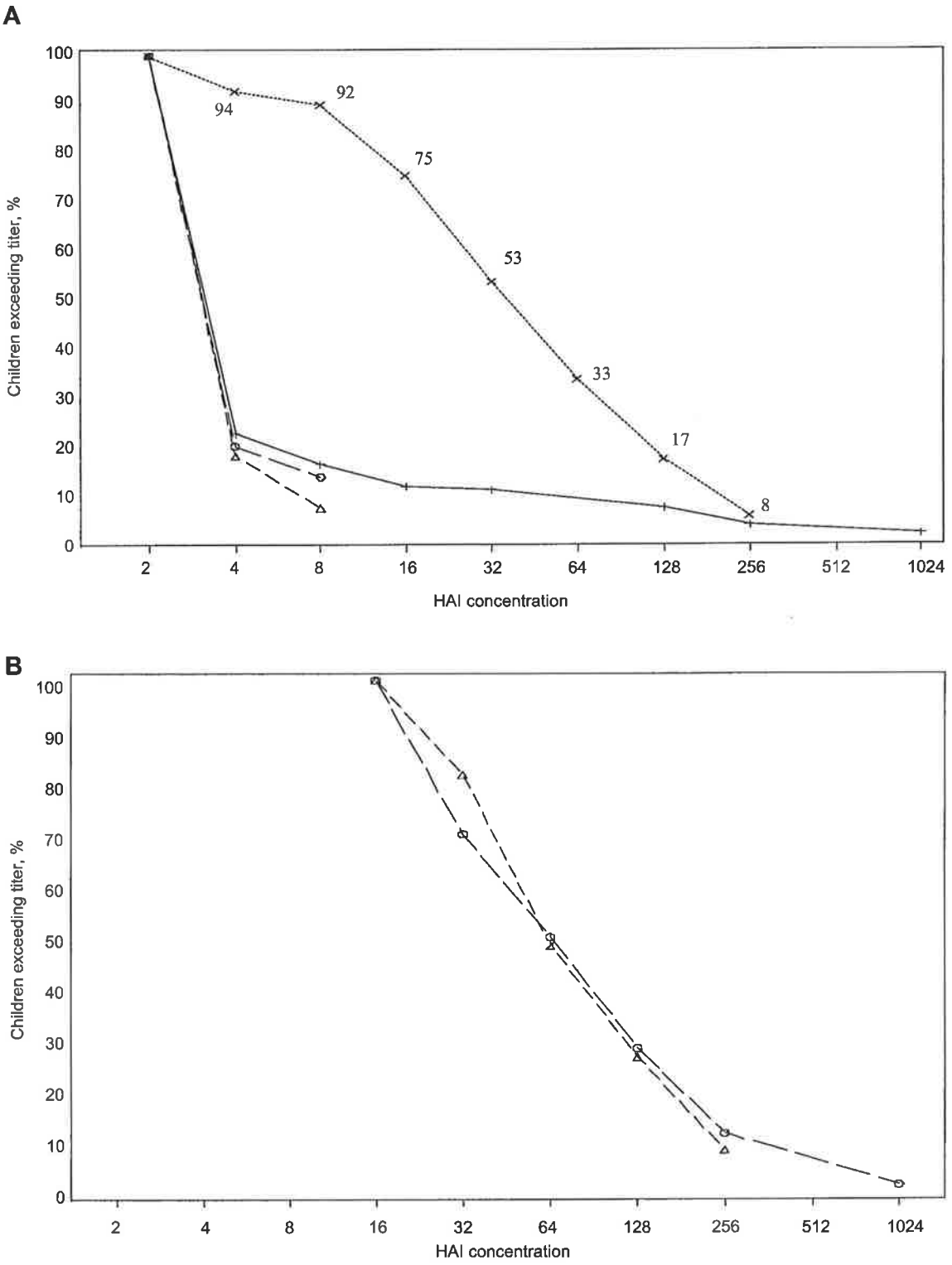

Figure 2. *A,* Reverse cumulative distribution curves of serum hemagglutination inhibition (HAI) antibody titers to parainfluenza virus 3 (PIV3) in seronegative children with paired serum samples available for analysis. Cumulative proportion of children achieving the indicated HAI titer is shown before and after vaccination with PIV3–cold passage mutant 45 (cp45) or placebo. Among 109 seronegative vaccine recipients and 110 placebo recipients, 86 (79%) and 13 (12%), respectively, had a 4-fold antibody increase ($P < .001$, Fisher's exact test). The postvaccine geometric mean antibody titer was 1:25 for vaccine recipients and <1:4 for placebo recipients. ○, Before PIV3-cp45; ×, after PIV3-cp45; △, before placebo; +, after placebo. *B,* Reverse cumulative distribution curves of serum HAI antibody to PIV3 for seropositive subjects. Prevaccination GMT was 1:50 for both vaccine and placebo groups and did not increase after vaccination. ○, PIV3-cp45; △, placebo.

Table 4. Serum hemagglutination inhibition (HAI) antibody response within 42 days (range, 35–56 days) after administration of parainfluenza virus 3 (PIV3)–cold passage mutant 45 (cp45) vaccine or placebo intranasally, by prevaccine antibody status.

	Prevaccine serum antibody status to PIV3							
	Seronegative (HAI ≤1:8)				Seropositive (HAI ≥1:16)			
Study group	No. of subjects tested	No. (%) of subjects with ≥4-fold increase in antibody titer	GMT to PIV3		No. of subjects tested	No. (%) of subjects with ≥4-fold increase in antibody titer	GMT to PIV3	
			Prevaccine	Postvaccine			Prevaccine	Postvaccine
PIV3-cp45	109[a]	86 (79)[b]	<1:4	1:25	60	2 (3)	1:50	1:46
Placebo	110	13 (12)	<1:4	<1:4	60	0 (0)	1:50	1:44

NOTE. GMT, geometric mean titer.

[a] Nos. of subjects with paired serum samples available are reported.

[b] $P < .001$, vs. placebo (Fisher's exact test).

uated nature of this PIV3 vaccine. Vaccination did not appear to confer significant protection against the causes of runny nose, cough, or fever during the 14 days after vaccination. Vaccination did not cause or protect against AOM or serous OM occurring within 42 days of vaccination. This result does not mean that the vaccine will not protect against PIV3-associated URI, LRI, or OM; rather, the occurrence of these illnesses caused by wild-type PIV3 during this short study was too infrequent to measure. Phase 3 studies are needed to measure these potential vaccine benefits.

PIV3-cp45 vaccine was immunogenic, and a single dose induced HAI antibody in 79% of seronegative (HAI titer ≤1:8) children. A single dose of vaccine increased serum PIV3 antibody to within 2-fold of preexisting, naturally acquired antibody titers observed in the seropositive subjects. In a previous study of PIV3-seronegative children, 18 (90%) of 20 children tested had ≥4-fold increases in HAI antibody titers, and 21 (100%) of 21 tested shed vaccine virus [15]. In contrast, seropositive children (titer ≥1:16) or adults shed vaccine virus less frequently than did seronegative children (2/16 [12%] seropositive children and 2/20 [10%] adults shed virus) and did not boost serum antibody titers (0/12 seropositive children and 0/10 adults had increases in antibody titers) [15]. Seropositive children 6–18 months old did not have increases in serum antibody titers in the present study. However, 2 doses of vaccine might improve the proportion of seronegative children who develop antibody after vaccination with PIV3-cp45.

In a previous study of this vaccine in infants 4–12 weeks old [15], 31 (94%) of 33 infants shed vaccine virus after dose 1 of PIV3-cp45 vaccine, but serum HAI antibody titers increased in the presence of maternal antibody (prevaccine geometric mean titer, 5.3 $\log_2$) in only 4 (13%) of 31 infants tested. A second dose of PIV3-cp45 vaccine given 1 or 3 months later resulted in vaccine virus shedding in 47% and 77% of infants, respectively, and only 1 infant (3%) had an HAI antibody response [15]. In contrast, IgA antibody to HN protein developed in more than one-half of the vaccinated infants after dose 1, and IgA levels were boosted in 47% and 66% of infants after dose 2 at 1 or 3 month intervals, respectively. These results are similar to the low frequency of serum antibody response observed in infants who are vaccinated with live attenuated RSV vaccines; multiple doses of live attenuated vaccine appear to be needed when infants are vaccinated in the face of maternal antibody and an immature immune system [17].

PIV3-cp45 vaccine is expected to prevent several significant clinical syndromes commonly caused by PIV3, including AOM, LRI, and febrile URI, but determination of efficacy will require an extended period of surveillance because PIV3 infections occur throughout the year [2–4]. An inl vaccine schedule to prevent LRI in infants, with vaccination at 1 month and again at 4 months, followed by inl boosting at 1 year, would be an attractive vaccine schedule to evaluate for efficacy, considering the results of the present phase 2 safety trial and those of the previous phase 1 trial in infants [15]. The addition of live attenuated RSV vaccine and, possibly, other live attenuated vaccines (PIV1, PIV2, and human metapneumovirus) would be a significant advance in controlling viral respiratory disease in young children, but these vaccines may not be available for several years. The safety and immunogenicity of PIV3-cp45 indicate that this vaccine should be evaluated for efficacy and effectiveness to help control this common cause of significant respiratory disease and AOM in infants and young children.

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MAJOR ARTICLE

Evaluation of Combined Live, Attenuated Respiratory Syncytial Virus and Parainfluenza 3 Virus Vaccines in Infants and Young Children

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We evaluated a combination respiratory syncytial virus (RSV) and parainfluenza 3 virus (PIV3) live, attenuated intranasal vaccine for safety, viral replication, and immunogenicity in doubly seronegative children 6–18 months old. RSV *cpts*-248/404 and PIV3-cp45 vaccines were combined in a dose of 10^5 plaque-forming units of each per 0.5-mL dose and compared with monovalent vaccines or placebo. The virus shedding pattern of RSV was not different between monovalent RSV *cpts*-248/404 vaccine and combination vaccine. Modest reductions in the shedding of PIV3-cp45 vaccine virus were found after the administration of RSV *cpts*-248/404 and PIV3-cp45 vaccine, relative to monovalent PIV3 vaccine; 16 (76%) of 21 children given combination vaccine shed PIV3-cp45 versus 11 (92%) of 12 of those given monovalent PIV3 vaccine. Both vaccines were immunogenic, and antibody responses were similar between the monovalent groups and the combination group. Combined RSV/PV3 vaccine is feasible for simultaneous administration, and further studies are warranted.

Respiratory syncytial virus (RSV) is the most important respiratory viral pathogen of infancy and childhood [1–3]. Most primary RSV infections are symptomatic [4, 5]; many RSV infections manifest as bronchiolitis and/or pneumonia, and severe infections occur in younger infants, with peak incidence of lower respiratory-tract disease (LRI) occurring in infants 2–6 months old, but a substantial portion of serious RSV illness and hospitalization still occurs in children >6 months old [6–9].

Human parainfluenza viruses (PIVs) are also important causes of serious respiratory-tract diseases in infants and young children <5 years old [10–12]. According to the Institute of Medicine, 25% of children <5 years old will have a clinically significant PIV infection annually, and ~2% will require hospitalization, most commonly for croup [12]. PIV is an important cause of or cofactor in acute otitis media (AOM) in children. Type 3 PIV (PIV3) is of particular significance in that, in addition to causing croup and bronchitis, it ranks second only to RSV as a cause of bronchiolitis and pneumonia in infants <6 months old. The virus causes severe disease throughout the first 2 years of life, and virtually all children have had primary PIV3 infections by 3–4 years of age. Overall, PIV3 is considered to be responsible for ~11% of hospitalizations for pediatric respiratory disease [11].

Significant progress is being made in developing live, attenuated intranasally (inl) administered vaccines for

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influenza, RSV, and PIV3 [13–17]. A cold-passage, temperature-sensitive strain of RSV (designated *cpts*-248/404) and a cold-passage, temperature-sensitive strain of PIV3 (designated -cp45) have been separately evaluated in phase 1 clinical trials in seronegative children 6–36 months old [15, 16]. Among children >6 months old, both vaccines were well tolerated and immunogenic at doses of 10^5 pfu and showed evidence of infection with vaccine virus (by isolation of virus and/or increase in antibody level) in >75% of children who were given inl vaccine [14, 16]. In studies of infants <6 months old who received 2 doses of PIV3-cp45 vaccine, there was evidence that the first dose provided some protection from shedding of vaccine virus after the second dose was administered, 1 month later [15]. Studies with PIV3-cp45 vaccine are progressing in infants [15], and a phase 2 study in 388 children 6–18 months old has confirmed the safety of this vaccine candidate for seronegative subjects [14]. The RSV candidate vaccine *cpts*-248/404 was evaluated in infants 4–12 weeks old and was found to cause nasal congestion that interfered with breast-feeding; therefore, additional attenuating mutations are being introduced into the vaccine [16]. However, for children >6 months old, RSV *cpts*-248/404 deserves further study.

In practice, it would be efficient to be able to administer these vaccines simultaneously. The present report summarizes the results of a phase 1 study evaluating the simultaneous administration of the RSV and PIV3 vaccines, each at a dose of 10^5 pfu, combined before inl administration. The objectives of the study were to (1) describe the infection rate, magnitude, and duration of shedding of RSV and PIV3 after 1 dose of a combined RSV/PIV3 vaccine administered inl; (2) determine the tolerability and safety of a combined RSV/PIV3 vaccine; (3) determine whether interference occurs when RSV and PIV3 are administered simultaneously; and (4) describe the antibody response as measured in serum and nasal-wash specimens.

SUBJECTS, MATERIALS, AND METHODS

Vaccines

The preparation and derivation of the RSV *cpts*-248/404 vaccine, a derivative of the A2 strain of RSV (subgroup A), and the PIV3-cp45 vaccine have been described elsewhere [15, 16]. RSV *cpts*-248/404 vaccine was prepared by Wyeth Vaccines at a titer of 1×10^6 pfu/mL. To achieve the planned dose for inoculation, this virus suspension was diluted in PBS with sucrose-phosphate-glutamate (PBS-SPG) to a titer of 4×10^5 pfu/mL. PIV3-cp45 vaccine was prepared by Wyeth Vaccines at a titer of 1×10^6 pfu/mL. Vaccine was diluted in PBS-SPG to a titer of 4×10^5 pfu/mL.

Combination vaccine was made by mixing equal volumes of the diluted monovalent vaccine, which resulted in titers of 2×10^5 pfu/mL for each vaccine strain. A dose, administered as 0.5-mL inl drops, delivered 10^5 pfu of each vaccine virus (RSV *cpts*-248/404 and PIV3-cp45). Monovalent vaccines were diluted to a titer of 2×10^5 pfu/mL, which resulted in a dose of 1×10^5 pfu/0.5 mL of nasal drops. Placebo consisted of PBS-SPG.

Study Design

The study was a multicenter, randomized, double-blinded comparison of bivalent vaccine, monovalent RSV *cpts*-248/404 vaccine, monovalent PIV3-cp45 vaccine, and placebo. Approximately 60 children, 6–18 months old, who were doubly seronegative for RSV and PIV3, were randomized in a 2:1:1:1 ratio to receive 1 of the following regimens: RSV *cpts*-248/404 and PIV3-cp45 combination vaccine (24 children), RSV *cpts*-248/404 monovalent vaccine (12 children), PIV3-cp45 monovalent vaccine (12 children), or placebo (12 children).

Study Subjects

Healthy children 6–18 months old whose parent(s) or guardian(s) gave informed consent to participate were enrolled. Children who were seronegative for RSV (defined as neutralizing antibody titer <1:40) and for PIV3 (defined as a hemagglutination inhibition [HAI] antibody titer ≤1:8) were selected. Children were screened by medical history and physical examination, to ensure that they had normal health and development. The human experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions were followed in the conduct of this clinical research.

Children were excluded if they had known or suspected impairment of immunological function or were receiving immunosuppressive therapy. Conditions for exclusion included systemic corticosteroid therapy, major congenital malformations, cytogenic abnormalities or serious chronic disorders, cardiac or respiratory illness, and any prior episode of wheezing confirmed by a physician (including illnesses diagnosed as asthma, wheezing, or reactive airway disease, whether attributed to environmental agents such as allergens or exposure to chemical irritants or to physical agents such as exercise- or cold-induced asthma, or infection). Also excluded were children with tympanostomy tubes and members of a household that contained a pregnant woman or an infant <6 months old or any immunocompromised individual. Children who attended day care and were in contact with infants <6 months old were excluded. They were also excluded if they exhibited a current febrile (temperature, ≥38°C) or other acute illness, including upper or lower respiratory symptoms (including nasal congestion that was considered significant enough to reduce the likelihood of successful immunization) or AOM at the time of enrollment. Infants born at <37 weeks gestation were deferred from study participation until they were at least 1 year old.

Procedures

Serum samples were obtained before inl vaccination and tested for antibody to RSV and PIV3, to select doubly seronegative children. Seronegative children were randomized to receive either a vaccine or the placebo by nose drops in a volume of 0.25 mL/nostril, for a total dose of 0.5 mL. The parents were asked to keep track of any illness or symptoms on a parent diary card each day for 14 days after vaccination and whenever the child felt warm during the 42-day postvaccine study period. Children were examined by study personnel, and nasal-wash samples were collected for the quantitation and phenotyping of shed virus on days 3–7, 8 or 9, 10, 12, 14, 17 or 18, and 28 after vaccination. These brief visits (20–30 min each) allowed the study staff to examine the child closely for any signs of a rhinorrhea, pharyngitis, fever, cough, respiratory illness, or ear infections. Six weeks after the first vaccination visit, all enrolled children returned to the clinic for a brief physical examination and a blood sample and nasal wash to measure antibody responses to vaccination.

Fever (rectal temperature, $\geq$38°C), upper respiratory-tract illness (rhinorrhea or pharyngitis), cough, and LRI were defined as described elsewhere [17]. AOM was defined as findings of inflamed, immobile tympanic membrane, with or without bulging, observed by a physician or nurse practitioner and confirmed by tympanometry or by a second observer. All children were examined at the end of the study, on days 35–49 after vaccination.

In the event of acute respiratory illness, additional nasal-wash samples were obtained and cultured for RSV, PIV3, and a variety of other common respiratory viruses, to help determine illness etiology. The nursing assessment completed during these visits included review of the diary card and transcription of the information, to determine the occurrence of any adverse events. A postimmunization blood sample and a nasal-wash sample were obtained from each child on day ~42 ($\pm$7 days) after immunization.

Laboratory Methods

Serum antibody response. Serum samples collected before and ~6 weeks after vaccination were evaluated for the presence of antibody to RSV and PIV3. Antibody to RSV was measured by the plaque reduction neutralization (PRN) assay, as described elsewhere [18]. Antibody to PIV3 was assessed by the HAI and by ELISA (IgA and IgG) to purified hemagglutinin-neuraminidase (HN) protein, as described elsewhere [19, 20].

Nasal-wash antibody response. Nasal-wash samples were evaluated by kinetic ELISA, as described elsewhere [21, 22], to determine the antibody response to RSV (glycoproteins F and Ga—i.e., the G attachment protein of RSV subgroup A) and to PIV3 (purified HN protein). The IgA concentration (in micrograms per milliliter) was determined similarly by use of a capture method. An increase in the ratio of $\geq$4 when prevaccination samples were compared with postvaccination samples from a child or seroconversion from 0 to any value was considered to be an increase in the level of nasal-wash antibody.

Vaccine virus quantitation and genetic stability. Vaccine virus shedding levels and temperature-sensitive *(ts)* phenotypic stability were determined from fresh or frozen nasal-wash samples by plaque assay. For the quantitation of RSV in nasal-wash samples, it was necessary to neutralize any PIV3 that might be present in the sample, to prevent any PIV3 cytopathic effect (CPE) from obscuring RSV plaques. Each sample to be tested for RSV was incubated for 1 h at 32°C in the presence of 5% anti-PIV3 horse serum, then serial 10-fold dilutions of PIV3-neutralized samples were inoculated onto HEp2 cell monolayers in duplicate 24-well plates and overlaid with 0.75% methylcellulose in minimum essential medium. The duplicate plates were incubated for 5 days at 32°C for virus quantitation or at 39°C for assessment of the *ts* phenotypic stability of the virus after replication within the human host. RSV plaques were stained in an indirect immunoperoxidase (IP) assay by use of RSV-specific monoclonal antibodies (MAbs) directed against F and G proteins and horseradish peroxidase (HRP)–labeled goat anti–mouse IgG, as described elsewhere [23]. Virus titers were expressed as $\log_{10}$ plaque-forming units per milliliter of nasal-wash fluid. To determine the *ts* phenotypic stability of the RSV recovered from the children, the infectivity titers of virus grown at 32°C and 39°C were compared. The *ts* phenotype of the RSV present in the sample was considered to be stable if the titer of RSV detected at 39°C was at least 100-fold lower that the titer of the RSV observed at 32°C.

The method to determine the titer and phenotype of the PIV3 in nasal-wash samples was modified from a plaque assay described elsewhere [24]. Plaques were visualized by use of PIV3-specific MAbs and HRP-labeled goat anti–mouse IgG in an indirect IP assay. Briefly, the agarose plugs were gently removed, and the monolayers were fixed with acetone:methanol (50%:50%). A mixture of PIV3 MAbs to the HN glycoprotein was added to each monolayer [25, 26]. After incubation for 1–2 h at 37°C or overnight at 2°C–8°C, the plates were washed with PBS, the substrate (Enhance Orange DAB-C/H_2O_2; Kirkegaard and Perry) was added, and the monolayers were incubated on a rocker platform for ~10–15 min at room temperature. The monolayers were rinsed with tap water and air-dried, and plaques were enumerated.

The genetic stability of PIV3 vaccine virus was assessed by measuring the *ts* phenotype of the shed virus in original nasal-wash samples at 39°C. Duplicate monolayers of LLC-MK2 cells were inoculated with test sample; 1 set was incubated at 32°C, and the other set was incubated at 39°C. The former was used to determine the titer of PIV3 shed by each child, and the latter was used to ascertain the genetic stability of the PIV3 being

recovered from the nasal-wash samples. The duration of viral shedding was defined as the last day of vaccine virus detection.

Diagnostic virologic testing on nasal-wash samples obtained during illness. Children who presented with respiratory symptoms during the study provided nasal-wash samples for virus isolation. Nasal-wash samples were collected and immediately diluted in 5× virus transport media. The samples were inoculated onto primary monkey kidney cells, as well as onto at least 2 other cells lines appropriate for the isolation of common respiratory viruses, including adenovirus, influenza A and B, PIV 1–4, and RSV. The tissue-culture cells were incubated at 32°C and observed for CPE for 14 days after inoculation.

If PIV3 was detected in association with illness and the result did not coincide with the detection of *ts* virus (vaccine) isolated from routinely scheduled nasal-wash samples, then the PIV3 isolate was identified by sequence analysis of reverse-transcription polymerase chain reaction (RT-PCR) products by use of primers specific for the amplification of a variable region of the F gene. The RT-PCR procedure used to detect wild-type *(wt)* PIV3 has been described elsewhere [15].

Statistical Analyses

Mean $\log_{10}$ titers of vaccine virus in nasal-wash samples were calculated for each day tested; nasal-wash samples in which virus was not detected (minimum amount of virus detectable, 0.7 $\log_{10}$ pfu) were considered to have a titer of 0.4 $\log_{10}$ pfu for our calculations. Days of virus shedding were compared between groups by use of the χ^2 text. Mean virus shedding in each monovalent group was compared with that in the combination vaccine group by use of analysis of variance (ANOVA).

RESULTS

A surprisingly high proportion of children screened, approximately one-half, were seronegative for both RSV and PIV3 at 6–18 months old. As expected, seronegative children tended to be younger than seropositive children. The average age of enrolled children was 10 months; 2 children were screened at 18 months of age but were not vaccinated until 19 months of age; they were included in the analysis. Fifty-four children were randomized—21 to the combined RSV *cpts*-248/404 and PIV3-cp45 group, 9 to placebo, and 12 each to RSV *cpts*-248/404 or PIV3-cp45 alone.

Shedding of RSV in the monovalent RSV vaccine group (figure 1*A*) and the combination vaccine group (figure 1*B*) was not significantly different in overall pattern, number of days of shedding (mean duration of RSV vaccine shedding, 14 days in the monovalent group and 15 days in the combination group; table 1), or mean peak titer shed (2.8 vs. 3.4 $\log_{10}$, monovalent vs. combination group; $P = .19$, ANOVA). In contrast, comparison of PIV3 shedding in the monovalent PIV3 group (figure 1*C*) and combination group (figure 1*D*) revealed modest viral interference, with PIV3-cp45 shedding in the combination group, compared with the PIV3 monovalent group. The mean peak titer of PIV3-cp45 shed was lower (3.8 vs. 2.1 $\log_{10}$, monovalent PIV3 vs. combination group; $P = .01$, ANOVA), and significantly fewer days of detection of PIV3-cp45 shedding were found in the combination group. The duration of PIV3-cp45 shedding (defined as the last day that vaccine virus was shed) was not significantly different between the 2 groups.

A total of 132 RSV-positive nasal-wash samples from 28 children and 123 PIV3-positive nasal-wash samples from 27 children were tested for *ts* phenotype. There was no change in the *ts* phenotype of RSV or PIV3 vaccine virus detected in postimmunization nasal-wash samples recovered from children enrolled in the study (data not shown.) These same samples contained RSV vaccine titers as high as 6.5 $\log_{10}$ pfu/mL or PIV3 vaccine titers as high as 5.4 $\log_{10}$ pfu/mL, which indicates multiple rounds of replication in the human nasopharynx without any change in the *ts* phenotype.

Clinical events occurring within 14 days of vaccination are summarized in table 2. LRI was not observed in any study subject. As expected, some children in the placebo group manifested fever, cough, rhinorrhea, or AOM, and these events reflect the high background rate of common upper respiratory-tract illnesses in young children. Although the rates of these minor illnesses did not differ significantly between vaccinees and placebo recipients, the study was not powered to examine the frequency of these events in placebo recipients versus vaccinees. However, these results provide background information for future studies to examine these questions; the sample size used in the present study has been used elsewhere [15, 16] to screen vaccine candidates and eliminate highly reactogenic or overly attenuated vaccines.

Children in the 3 vaccine groups manifested a similar spectrum of illnesses as the children who received placebo, with the possible exception of the occurrence of AOM. Evidence of AOM was observed in 4 (38%) of 12 children vaccinated with RSV *cpts*-248/404 (3/4 children with AOM shed a virus other than vaccine type, including 1 each of *wt* PIV, adenovirus, and influenza virus) and 7 (33%) of 21 children given the combination of RSV *cpts*-248/404 and PIV3-cp45 vaccines (0/7 shed a *wt* virus), compared with 1 (8%) of 12 in the PIV3-cp45 group (this child shed influenza A virus and PIV3-cp45 vaccine) and 1 (11%) of 9 in the placebo group. However, these rates were not statistically different. Other manifestations of viral respiratory disease were common in all study groups, including cough, rhinorrhea, and fever.

In addition to the concurring viral infections associated with AOM, as described above, concurrent viral infections were detected in study children and included 1 *wt* RSV infection in a child in the monovalent PIV3-cp45 vaccine group and 2 children with enterovirus infections in the combination vaccine group. *wt* PIV3 was circulating at some of the study sites at

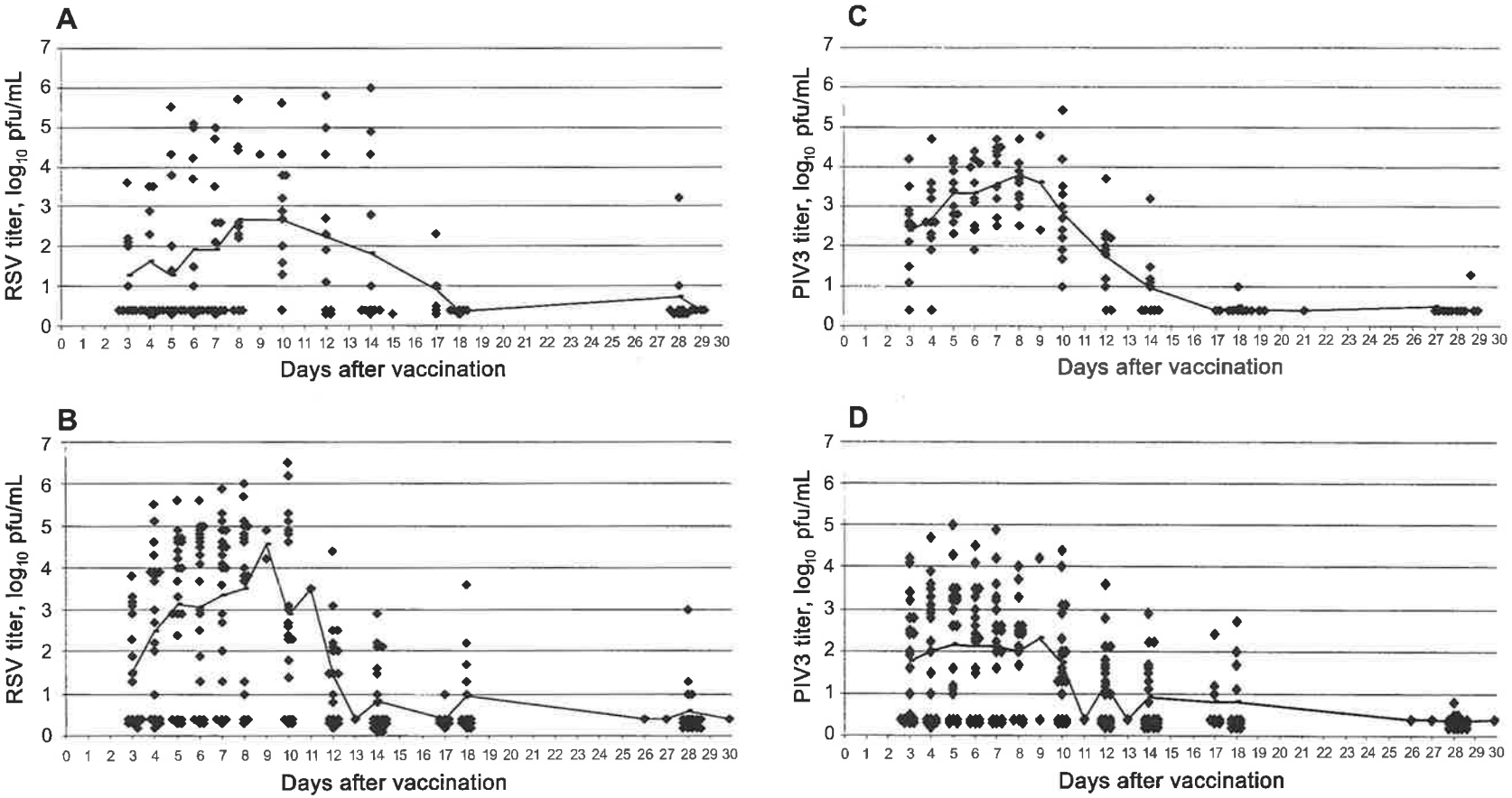

Figure 1. Virus shedding pattern of respiratory syncytial virus (RSV) *cpts*-248/404 in the monovalent RSV vaccine group *(A)* vs. the virus shedding pattern of RSV *cpts*-248/404 in the combination RSV/parainfluenza 3 virus (PIV3) vaccine group C *(B)* ($P = .19$, analysis of variance [ANOVA]). Virus shedding pattern of PIV3-cp45 in the monovalent PIV3 vaccine group *(C)* vs. the virus shedding pattern of PIV3-cp45 in the combination RSV/PIV3 vaccine group *(D)* ($P = .01$, ANOVA).

Table 1. Mean duration of virus shedding and mean quantity shed on the peak day of virus shedding of respiratory syncytial virus (RSV) *cpts*-248/404 or parainfluenza 3 virus (PIV3)–cp45 vaccine among children intranasally given 10^5 pfu of monovalent RSV vaccine, 10^5 pfu of monovalent PIV3 vaccine, 10^5 pfu each of RSV and PIV3 vaccines, or placebo.

Group	No. shedding/ no. vaccinated (%)		Duration of shedding,[a] mean, days		Virus titer on peak day of shedding,[b] mean, pfu/mL $\log_{10}$	
	RSV	PIV3	RSV	PIV3	RSV	PIV3
RSV *cpts*-248/404	11/12 (92)	...	14	0	2.8	<0.5
PIV3-cp45	...	11/12 (92)	0	15	<0.5	3.8
RSV *cpts*-248/404 and PIV3-cp45	19/21 (90)	16/21 (76)	16	16	3.4	2.1[a]
Placebo	0/9	1/9[c]	0	0	<0.5	<0.5

[a] Significantly more days with no shedding of PIV3-cp45 were seen in the combination group vs. the monovalent PIV3-cp45 group. Duration of shedding was calculated by use of the last day of virus isolation to indicate the total duration of virus replication.

[b] Mean virus titer on peak day was calculated by use of the peak titer of virus shed from each child on day 10 for the RSV monovalent group and on day 7 for the PIV3 monovalent group and the RSV/PIV3 combination group.

[c] Wild-type PIV3 was isolated from 1 control child.

the time of the study, and evidence of concurrent RSV or PIV3 infection was present in the serologic results. Specifically, 2 children in the PIV3 group had antibody increases to RSV, and antibody increases to PIV3 occurred in 2 children who received RSV vaccine and in 3 placebo recipients.

Both the RSV and PIV3 vaccine strains induced the production of serum and mucosal antibody. The frequency of developing serum neutralization antibody responses for RSV or serum HAI antibody responses for PIV3 was not significantly different in the monovalent groups versus the combination group (table 3). The RSV vaccine above induced antibody in 9 (90%) of 10 children, and the RSV/PIV3 combination vaccine induced RSV antibody in 18 (95%) of 19 children. There was a suggestion that PIV3 immunogenicity was reduced, but this did not achieve statistical significance; 9 (82%) of 11 children developed antibody in the monovalent PIV3-cp45 group, compared with 12 (60%) of 20 in the bivalent group ($P = .26$). Among those who were infected by a vaccine virus, the post-vaccine geometric mean PRN antibody titer to RSV or HAI antibody to PIV3 was not significantly different (table 3, footnotes c and d). Nasal-wash antibody responses to RSV for Ga antigens occurred in 6 (50%) and 8 (67%) of 12 children, respectively, in the monovalent vaccine group and in 7 (33%) and 14 (67%) of 21 children, respectively, in the combination vaccine group (table 3). After a single dose of vaccine, nasal-wash antibody response to PIV3 was found in only 2 (17%) of 12 children in the monovalent vaccine group and in 6 (29%) of 21 children in the combination group.

DISCUSSION

The development of a safe and effective vaccine for the prevention of respiratory disease caused by RSV and PIV3 represents an important but elusive objective. RSV is the most important cause of LRI in infants and young children, and it causes significant disease in elderly and immunocompromised patients. PIV3 is

Table 2. No. of children with the indicated sign or symptom of illness on days 0–14 after intranasal vaccination with respiratory syncytial virus (RSV) *cpts*-248/404, parainfluenza 3 virus (PIV3)–cp45, a combination of both vaccines, or placebo.

Group (no. of children)	Temperature ≥38°C	Cough	Rhinorrhea	LRI	AOM
RSV *cpts*-248/404 (12)	2	7	11	0	4[d]
PIV3-cp45 (12)	1[a]	4	8	0	1[e]
RSV *cpts*-248/404 and PIV3-cp45 (21)	7[b]	7[c]	19	0	7[f]
Placebo (9)	4	3	5	0	1

NOTE. Data are no. of children. AOM, acute otitis media; LRI, lower respiratory-tract disease.

[a] One child with temperature ≥38°C shed wild-type *(wt)* RSV.

[b] Four children with a temperature ≥38°C shed both RSV and PIV3 (2 of the 4 also shed an enterovirus), and the other 3 shed only the RSV vaccine phenotype.

[c] Four children with cough shed RSV and PIV3, 2 shed RSV only, and virus was not isolated from 1.

[d] Concurrent *wt* infections occurred in 3 children—1 each of PIV, adenovirus, and influenza virus.

[e] Concurrent infection with influenza A occurred in this child.

[f] The children with fever, cough, and/or AOM were not the same 7 children; 14 children had various combinations of fever and/or cough and/or AOM.

Table 3. Development of ≥4-fold serum and/or nasal-wash antibody responses to respiratory syncytial virus (RSV) or parainfluenza 3 virus (PIV3) among initially seronegative children after vaccination with RSV *cpts*-248/404, PIV3-cp45, both vaccines, or placebo.

	Increase in serum antibody, no. with increase/no. tested			Increase in nasal-wash antibodies, no. with increase/no. tested			
Group (no. of children)	RSV	PIV3	Both	RSV F	RSV Ga	PIV3	Both
RSV *cpts*-248/404 (12)	9/10	2/11[a]	1/10[a]	6/12	8/12	1/12	0/12
PIV3-cp45 (12)	2/12[a]	9/11[b]	2/11[a]	3/12	1/12	2/12	0/12
RSV *cpts*-248/404 and PIV3-cp45 (21)	18/19[c]	12/20[b,d]	11/18	7/21	14/21	6/21	5/21
Placebo (9)	0/5	3/9[a]	0/5	2/8	0/8	3/8	0/8

NOTE. Seronegative to RSV, serum neutralizing antibody titer <1:40; seronegative to PIV3, serum hemagglutination inhibition (HAI) antibody ≤1:8.

[a] Wild-type RSV unexpectedly circulated in the community, and PIV3 was endemic, as expected.

[b] Nine of 11 vs. 12 of 20 ($P = .26$).

[c] Among those who were infected with RSV vaccine-type virus, as indicated by shedding of RSV *cpts*-248/404 or ≥4 fold antibody increase to RSV, the log mean postvaccine neutralizing antibody to RSV was not significantly different in the monovalent vs. combination group, 8.8 ± 1.1 vs. 8.1 ± 1.0. Geometric mean titers were 504 and 276, respectively.

[d] Among those who were infected with PIV3 vaccine virus, as indicated by shedding of PIV3-cp45 or ≥4 fold antibody increase to PIV3, the log mean postvaccine HAI titer to PIV3 was not significantly different in the monovalent vs. combination group, 4.1 ± 1.7 (±SD) vs. 3.6 ± 2.0; geometric mean HAI antibodies were 17 vs. 12, respectively.

the second most important cause of bronchiolitis and pneumonia during the first 6 months of life and is a common cause of febrile respiratory disease and AOM in older children. Recent experience with a cold-adapted, *ts*, attenuated influenza vaccine administered by the inl route demonstrated the feasibility of this technology for vaccine administration [13].

Several key issues in developing a bivalent RSV-PIV3 vaccine were addressed in the present pilot study. We sought to develop preliminary observations to assess whether combined vaccine would exhibit evidence of augmented reactogenicity. RSV and PIV3 frequently are involved in dual or mixed viral infections that occur in ~15% of respiratory illnesses in the community [27–30]. In general, the clinical syndromes associated with dual respiratory viral infections have appeared to be indistinguishable from single-agent infections, although one review [29] found that dual infections may be more severe and were more likely to result in hospitalization. Additionally, evidence of viral interference or decreased immunogenicity was sought to determine a preliminary strategy for the vaccination of children against both diseases.

The present results suggest that bivalent RSV/PIV3 vaccine is feasible to develop. A majority of children in the bivalent vaccine group responded to both the RSV and PIV vaccine components. The present results demonstrate only very modest interference by RSV with the PIV component. A simple strategy to overcome this interference would be to give 2 doses separated by an appropriate interval, to be determined by clinical investigations—a 2-month interval was successful with live, attenuated trivalent inl influenza vaccine. In previous studies in this age group [16], the RSV *cpts*-248/404 vaccine appeared to be satisfactorily attenuated, but it retained some reactogenicity in infants <6 months old. As a test-of-concept study, the acute safety of the combined RSV-PIV3 vaccine could not be differentiated from those of either monovalent vaccine or placebo. Although no significant differences were observed, the clinical events associated with the RSV component of bivalent vaccine may be significant, but confirmation of this observation will require a larger study, given the high frequency of concurrent virus infections in this age group.

Peak virus shedding titers in the monovalent RSV *cpts*-248/404 vaccine group were higher than have been previously reported [16]; the higher titers in the present study reflect a change in the laboratory assessment methods. In the present study, IP staining of plaques was used to determine titers, whereas, elsewhere, plaque assay without IP was used. Similarly, peak virus shedding titers in the monovalent PIV3-cp45 vaccine group were higher than those reported elsewhere because of our use of an IP stain to determine plaque count [15, 17].

The characteristics of PIV3-cp45 vaccine appear to be suitable for expanded trials. Recently, a phase 2 study was completed in 380 children 6–18 months old. No increase in rhinorrhea, cough, fever, or AOM was found when these findings were compared in seronegative, vaccinated recipients versus placebo control subjects. PIV3-cp45 vaccine induced vigorous HAI antibody responses (geometric mean titer [GMT], 1:24 after vaccination) that were within ~2-fold of the antibody level found in naturally infected children before vaccination (GMT, 1:50).

Genetic stability was assessed, and the vaccine viruses retained their *ts* phenotype, despite multiple cycles of replication in young seronegative children; the multiple genetic changes introduced into PIV3-cp45 and RSV *cpts*-248/404 provide a good means of safety to ensure that viruses with a virulent phenotype will not emerge during replication in children.

Significant progress is being made toward further attenuation

of the RSV component. Once a suitable RSV vaccine component is derived, it can be combined with PIV3-cp45 vaccine and evaluated for the safety, infectivity, and efficacy of each component. The present study has provided a rationale and a model protocol for proceeding with those future evaluations. The RSV *cpts*-248/404 component interfered with PIV3-cp45, but not the reverse; this suggests that further attenuated derivatives of RSV *cpts*-248/404 might not interfere or would interfere to a lesser degree and, therefore, would work well in combination with PIV3-cp45, but this will need to be tested in clinical trials and determined empirically. The results of the present test-of-concept trial provide the framework for future development of bivalent RSV/PIV3 vaccine.

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Chapter 3: Community and Immunisation Provider Acceptance of New Vaccines

This chapter describes the results of studies conducted to assess community and immunisation provider awareness and acceptance of new vaccines once they become licensed in Australia. Once a vaccine is licensed a recommendation can be made by the Australian Technical Advisory Group on Immunisation (ATAGI) for the vaccine to be federally funded. Decisions on funding and inclusion of a newly licensed vaccine into the National Immunisation Program (NIP) are then made by the Pharmaceutical Benefits Advisory Committee (PBAC) and depend substantially on the results of cost effectiveness analyses including the cost of the immunisation program and the cost saving in prevention of the associated morbidity and mortality from the disease.

Therefore, there is often a delay in provision of these vaccines to the public even with an efficient process. Often, initial availability is only in the private market at a high cost, resulting in inequality in access to these vaccines with the consequence that vulnerable children remain without protection. Varicella vaccine was recommended by ATAGI but only available on the private market in Australia for five years before it was funded for all children. Not only does this result in a high proportion of children at risk of varicella and its complications, but low coverage with a live attenuated vaccine such as varicella, has implications for a potential change in epidemiology of the disease in the community with a potential increase in adult disease. Our study reports on the uptake of varicella vaccine in the community prior to funding for the vaccine being provided.

Human Papillomavirus (HPV) vaccine was recently introduced for adolescent girls with a catch-up program for young women to provide protection against cervical cancer. Not only is the vaccine likely to provide protection against cervical cancer but also against other HPV related cancers such as anal and other genital cancers including vulval, vaginal and penile cancer and oro-pharyngeal cancers. There is obviously benefit to for adolescent boys to receive the vaccine as well both to protect themselves against HPV related cancers but also to contribute to the herd immunity effect of reducing exposure to the virus in the community.

The Australian of the Year, Professor Ian Frazer raised the level of awareness of the association between HPV and cervical cancer, not only in the community but particularly in the media following licensing of the HPV vaccine. In response, issues related to implementation of a HPV immunisation program, such as

concern about promiscuity in adolescent girls were raised in the media and by some religious groups. Our study investigates acceptance of the vaccine in the community prior to introduction of the vaccine.

The recent H1N1 pandemic and previous H5N1 outbreaks have resulted in the manufacture of many new influenza vaccines for control of pandemic infection. Acceptance of these vaccines by the community and an appreciation that once available vaccine delivery may be prioritized to certain groups in addition to other infection control strategies is essential to control the spread of infection during a pandemic. Our study examined community awareness and acceptance of strategies to prevent the spread on infection during a pandemic.

Providing accurate, transparent information and education to the community prior to introduction of a vaccine with an efficient funding review process is essential to ensure optimal uptake and protection for the community.

Introduction of varicella vaccine (recommendation without funding)

8. Marshall H, Ryan P, Roberton D. Uptake of varicella vaccine – a cross sectional survey of parental attitudes to nationally recommended but unfunded varicella immunisation. Vaccine. 2005;23:5389-97.

Although varicella vaccine was approved in Australia for use in children from 12 months of age by the National Health and Medical Research Council (NHMRC) in 2000, recommendation for use of the vaccine and incorporation into the Australian Standard Vaccination Schedule (ASVS) only occurred in September 2003. However, at the time this study was conducted, Government funding for the vaccine had not been provided and purchase of the vaccine was at parental expense. Varicella vaccine uptake had been slow, resulting in incomplete coverage compared to federally funded vaccines.

Low varicella vaccine coverage in the community has particular implications not seen with other vaccines. When only a proportion of the population is immunised there is less contact with wild type infection in infancy and early childhood. While varicella continues to spread within the community, there is a higher risk of developing the disease at an older age when the disease is more serious and more costly to the

community. A high coverage rate in the population (at least 75%) has many benefits including a significant reduction in exposure to disease and potentially a reduction in the incidence of herpes zoster later in life.

Paper 8 describes the results of a study to assess uptake of a varicella vaccine in the community prior to Federal funding being provided and inclusion in the National Immunisation Program. Our study confirmed that there was inadequate varicella vaccine coverage in children in South Australia prior to funding. This was particularly evident in the 9 month to 4 years age group who experience the highest rates of hospitalisation for varicella infection. We were particularly interested in identifying the reasons for poor uptake in the community.

This study provided the first Australian data on reasons why parents choose whether or not to immunise their children against varicella infection. The main reasons reported in our study for not having children immunised with varicella vaccine were related to lack of funding and knowledge about the vaccine rather than concerns about the vaccine or associated side effects. The three most commonly cited reasons (excluding previous varicella infection) for not immunising a child were due to lack of knowledge about the vaccine, lack of awareness that the vaccine was included on the ASVS, and the cost of the vaccine to families. Most caregivers identified prevention of disease as the primary reason for immunising their child. Others identified their GP as having a strong influence on whether their child received recommended vaccines.

Our study showed that barriers to varicella immunisation are the result of poor knowledge about the vaccine and lack of funding. Recommending a vaccine without providing funding, gives "mixed messages" to immunisation providers, parents and caregivers. The minority of parents that were unaware that a varicella vaccine was available is of concern. The study results suggest that parents were not well informed about the vaccine and parental education needs to be a significant component of any new immunisation campaign to increase coverage. However, the most important consideration in ensuring optimal uptake of a new vaccine in the community is the provision of Government funding for new vaccines once they become licensed.

Data from this study were used by ATAGI in recommending funding of varicella vaccine. Funding was provided in November 2005.

I presented the results of this study at the Australian Society for Infectious Disease (ASID) conference at Margaret River, Western Australia in August 2005 and the 36th Public Health Association of Australia conference in Perth, Western Australia Australia, September, 2005.

9. Marshall H, Ryan P, Roberton D, Beilby J. Varicella immunisation practice: Implications for provision of a recommended, non-funded vaccine. Journal of Paediatrics and Child Health. 2009;45:297-303.

As our previous study indicated that GP recommendation was an important factor in determining acceptance of recommended, non-funded vaccines by parents, we aimed in the study described in Paper 9 to establish the factors that increased the likelihood of non funded vaccines being recommended by GPs. Although the number of paediatric consultations by a GP appeared to be strongly associated with provision of a non-funded vaccine such as varicella, to children at routine immunisation visits, other factors were important in acceptance by parents, including provision of information about the vaccine and the wish by parents to prevent disease.

Our study showed that GPs who had recently graduated were more likely to recommend new, non funded vaccines possibly due to recent graduates having received more intensive teaching and training on immunisation, with administration of newer vaccines considered important components of preventive health care. We showed that female GPs were more likely to discuss non-funded vaccines with their patients which may be due to longer time spent in consultation with a patient compared with consultation time with a male GP. Discussion about non-funded vaccines requires additional time and assessment and may be given less priority than discussion about funded vaccines by GPs because of less emphasis on vaccination with these vaccines from state immunisation authorities.

Recommending a vaccine without providing funding is likely to result in variability in recommendations to parents from GPs and GP judgement of parental affordability of the vaccine. Our study findings suggest that when parents are provided with information about the vaccine, they are more likely to accept their GP's recommendation. Provision of information about the vaccine is likely to instil confidence in parents that an informed decision has been made to have their child immunised. Our study suggests that provision of a fact sheet summarising information about the vaccine preventable disease and the vaccine at the time of

patient booking could improve the decision-making process for the parent without impacting significantly on consultation time.

Recent changes to the recommendations for varicella immunisation include receipt of two doses of varicella-containing vaccine to provide increased protection and minimise break through disease in children. However, routine administration of a second dose of varicella-containing vaccine is not included on the NIP and was rejected by the Pharmaceutical Benefits Advisory Committee following recommendation by the ATAGI because of the lack of evidence of cost-effectiveness of a second dose. We can predict that provision of a second dose to all children will be low. Education of GPs about recommendations to improve protection of children is of paramount importance, particularly when funding of an optimal vaccination program is not guaranteed.

Introduction of Human Papillomavirus vaccine

10. Marshall H, Ryan P, Roberton D, Baghurst P. A cross-sectional survey to assess community attitudes to introduction of Human Papillomavirus vaccine. Australian and New Zealand Journal of Public Health. 2007;31(3):235-242.

HPV infection is the undisputed cause of cervical cancer with approximately 20 high risk oncogenic strains having been shown to be responsible for the majority of cases. Although women are at risk of acquiring the virus and developing cervical cancer, both men and women may transmit the virus to their partner during sexual activity.

Vaccines against the high-risk types HPV-16 and HPV-18 have been shown to be safe and immunogenic in previous trials, and have been shown to prevent incident and persistent HPV-16/18 infection and cervical intraepithelial neoplasia (CIN) I, II and III.

Two HPV vaccines have recently been licensed in Australia with funding provided for vaccination of adolescents and a catch-up program for young women. Adolescent vaccination is important prior to onset of sexual activity and exposure to oncogenic HPV strains to provide optimal protection. Community acceptance of vaccination of young adolescent girls before they become sexually active is paramount for

successful immunisation programs. My interest in community acceptance of HPV vaccine arose from conducting a multicentre safety and immunogenicity study of HPV vaccine in adolescent girls, 10-14 years of age. The results of this study have been outlined in a manuscript yet to be submitted for publication.

Paper 10 outlines the results of this community study which aimed to assess knowledge and community attitudes in both men and women to the introduction of HPV vaccines in metropolitan and rural South Australia. This was the first quantitative study to investigate men's in addition to women's understanding and acceptance of HPV vaccine.

Prior to this study being conducted concerns had been raised in the media in Australia and the US about the social implications of vaccinating adolescents to prevent a sexually transmitted disease and potentially cervical cancer. The implication that cervical cancer is linked to a sexually transmitted disease may lead to anxiety and concern about the use of HPV vaccine.

Our study indicated that although there is a high acceptance of HPV vaccination in the community, knowledge about the causal relationship between HPV infection and development of cervical cancer is deficient, particularly for men, despite a high level of acceptance of the vaccine. Our results indicated that education about HPV infection and prevention particularly needs to be directed towards men, young adults and the elderly, those with lower educational attainment and those who are the most disadvantaged in the community.

The acceptance of a vaccine to prevent a sexually transmitted infection and ultimately cancer in the South Australian community was established by our study results. Despite poor knowledge about the cause of cervical cancer the majority of adults and parents are willing to accept vaccination to prevent this disease, with acceptance of vaccination being only slightly higher in females than in males.

Our results confirmed that parents are not concerned about discussing sexually transmitted disease with their children and were willing to discuss use of the vaccine at an appropriate age. There was little evidence in our study results to suggest that anxiety about use of the vaccine leading to promiscuity is a concern amongst parents.

Understanding community concerns is essential when developing education campaigns prior to vaccine delivery. This study provided information for educators and policy makers prior to the introduction of a HPV vaccination program.

Linkages between health care and education systems to provide education about the benefits and availability of the HPV vaccine will be vital to the achievement of high levels of coverage. Implementation issues including provision of Government funding, decisions on whether or not both males and females will receive the vaccine and the target age group are yet to be determined. Education of both men and women will be essential to ensure the advantages of herd immunity in communities.

This study was supported by a scholarship I received as the inaugural recipient of the Public Health Education and Research Trust (PHERT) Scholarship. This competitive award was granted to me in recognition of the importance of establishing data on the community acceptance of the HPV vaccine prior to commencement of the HPV immunisation program. I received numerous invitations to present these study data at national meetings due to the high level of interest from policy makers in the study findings.

I presented these study results at the 10th National Public Health Association of Australia Immunisation Conference/2nd Asia Pacific Vaccine Preventable Diseases Conference in Sydney, New South Wales, Australia, July 30 – August 1, 2006 and was invited to present the study results as the recipient of the PHERT award at the 37th Public Health Association of Australia Conference in Sydney, New South Wales, Australia, September 25 – 27, 2006.

Acceptability of pandemic influenza vaccines and other preventative strategies

11.Marshall H, Ryan P, Roberton D, Street J, Watson M. Pandemic Influenza and Community Preparedness. American Journal of Public Health 2009;99:S365-71.

The recent H1N1 pandemic and H5N1 outbreak have led to the production of pandemic influenza (PI) vaccines by almost all vaccine manufacturers. Although this will result in billions of doses of vaccine being available not everyone will have access to these vaccines and governments will decide who in the community will have priority access to these vaccines. In addition, strategies such as home isolation,

wearing of masks, use of anti-viral medications and school closures have all been initiated in the current pandemic. Our study was conducted prior to the onset of the H1N1 pandemic to assess community knowledge of PI preparedness and acceptance of government strategies including vaccination to prevent the spread of infection.

Our study showed that despite poor knowledge, there is a high level of concern about PI within the community, particularly amongst elderly women and adults in low income households possibly due to perceived vulnerability due to lack of resources, potential loss of income and concern for dependents. Education about PI was shown to be deficient which needs to be addressed for successful community engagement in PI preparedness plans. However acceptance of vaccination as a strategy to prevent spread of infection once a licensed vaccine was available was supported by the community, including the suggestion of compulsory vaccination being considered in this emergency situation. The most vulnerable groups within society including children, the elderly and those who are unwell were selected by the community as a priority for vaccination. Children have also been considered an important priority for vaccination by government, to control the spread of infection, including reduction in transmission to the elderly. Our results showed that although the majority would agree to be vaccinated, almost 12% would refuse vaccination equating to 2.4 million individuals in Australia who would remain unprotected with the potential to spread infection.

Our study results highlight the importance of educating the community prior to the onset of an influenza pandemic and engaging the community in pandemic influenza preparedness plans to increase awareness and acceptance of strategies to reduce the spread of infection in the community.

I presented the study results at national and international meetings including the 5th World Congress of the World Society for Pediatric Infectious Diseases (WSPID) in Bangkok, Thailand, November 15 – 18, 2007 and the 13th International Congress on Infectious Diseases (ICID) in Kuala Lumpur, Malaysia in June 19 – 22, 2008 and the 11th National Public Health Association of Australia (PHAA) Immunisation Conference in Surfers Paradise, Queensland, Australia, September 16 – 18, 2008.

Compulsory Immunisation: risks and benefits

12. Isaacs D, Kilham H, **Marshall H.** Should routine childhood immunizations be compulsory? Journal of Paediatrics and Child Health 2004;40:392-396.

This paper was written with co-authors after I completed an elective topic "Ethics in Public Health" during my Master in Public Health Degree, but was not a requirement of the course or submitted towards my degree. This review paper provides an ethical and historical view on whether immunisation should be compulsory in Australia.

This paper outlines the benefits and risks involved in immunisation and the importance in Australian culture for free parental decision regarding whether or not to have a child immunised. We either choose a paternalistic approach and make immunisation compulsory or we accept parental autonomy in making the decision whether or not to have their children immunised. Compulsory immunisation is regarded by some as justifiable in terms of the benefit to the individual and to the community, particularly when there is a threat to the community as demonstrated in the previous paper (Paper 11). However compulsory immunisation infringes the autonomy of parents to make choices about child rearing, which is an important consideration in our society. There are also practical considerations in enforcing immunisation, such as restraining children without parental consent, or fining parents, which are unlikely to be acceptable in our society. Alternatives to compulsory immunisation that have been successful in Australia and have achieved high coverage include inducements, e.g. linking child care and maternal benefits to immunisation, school requirements for knowledge of and recording of immunisation status and emergency legislation to compel immunisation in the face of an outbreak or pandemic such as we are currently experiencing.

We conclude that children should not be compulsorily immunised when high coverage rates can be achieved with education and inducements. With the current high levels of coverage at around 92% for childhood vaccinations, compulsory vaccination is not required. The case might be stronger if immunisation coverage levels fell, although this would likely result in an increase in epidemics of vaccine-preventable infectious diseases with public recognition of the value of immunisation and hopefully improved immunisation rates without the need for compulsion.

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Uptake of varicella vaccine—a cross sectional survey of parental attitudes to nationally recommended but unfunded varicella immunisation☆

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Abstract

The aim of this study was to assess the uptake of varicella vaccine in South Australian children under circumstances where varicella immunisation is recommended, but is not funded by Government. The study examined the main reasons that determined a parent's decision whether or not to have their child immunised with varicella vaccine. A cross-sectional survey was conducted by Computer Aided Telephone Interviews (CATI) in June 2004. Data were obtained from 613 households containing 1148 children aged from birth to 17 years of age. Statistical analyses were performed using data weighted to the South Australian population. Six hundred and eighty children (55.7%) had a history of varicella infection and 446 children (42.0%) had received varicella vaccine (weighted data). The most common reasons cited for not having children immunised included lack of knowledge about the vaccine and cost. One year after inclusion of varicella vaccine in the Australian Standard Vaccination Schedule there is evidence of incomplete coverage in children in South Australia due to absence of government funding for vaccine provision.

Keywords: Varicella; Vaccine uptake; Parental attitudes

1. Introduction

There are approximately 240,000 cases of varicella each year in Australia, resulting in 1500 hospitalisations and 10–20 deaths [1,2]. In healthy children, varicella is usually a mild disease, but in adults and immunocompromised people of any age there may be severe complications including encephalitis or pneumonia. The mortality rate in immunocompromised individuals is 7–10% compared with 0.1–0.4% in healthy children [3]. The highest rates of hospitalisation occur in children under 4 years of age [1].

Although varicella vaccine was approved in Australia for use in children from 12 months of age by the National Health and Medical Research Council (NHMRC) in 2000 [4], recommendation for use of the vaccine and incorporation into the Australian Standard Vaccination Schedule (ASVS) only occurred in September 2003 [5]. However, Government funding for the vaccine has not been provided and purchase of the vaccine is at parental expense.

In Australia, varicella vaccine uptake has been slow, resulting in incomplete coverage compared to Federally funded vaccines. According to data from the Australian Childhood Immunisation Register (ACIR, 2004), 91.2% of South Australian children have received the Federally funded vaccines included on the National Immunisaton Program by 12 months of age; 92% have received DTPa, 91.8% have received polio, 95.1% have received *Haemophilus influenzae* and 95.4% have received Hepatitis B vaccine. By 2 years of age 94.3%

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of children have received the Federally funded measles, mumps, rubella vaccine. Ideally, a coverage rate similar to that achieved with funded vaccines should be attainable once varicella vaccine becomes funded. Previous estimates of varicella vaccine coverage in Australia have relied on ACIR data and sales figures from distribution of vaccines nationally. An annual uptake of approximately 8% in children 1–4 years of age has been calculated from South Australian sales figures of Varilrix® vaccine in 2003 (GlaxoSmithKline Australia (GSK)). Comparable data from the ACIR suggest a 4.5% uptake in this age group in 2003 and a 6% uptake during 2004. However, estimation of coverage from ACIR data is likely to be inaccurate due to underreporting. General Practitioner (GP) incentive payments require notification of administration of funded vaccines to the ACIR. As varicella is not yet funded there is less incentive for notification by GPs.

The cost of varicella vaccine is an obvious deterrent at $50.00 to $75.00 per dose. Concern about perceived side effects may also contribute to low uptake of the vaccine [6]. A partially immunised community is of concern because of the induced changes in epidemiology of the disease [7]. When only a proportion of the population is immunised there is less contact with wild type infection in infancy and early childhood. While varicella continues to spread within the community, there is a higher risk of developing the disease at an older age when the disease is more serious and more costly to the community [8–12].

A high coverage rate in the population (at least 75%) has many benefits including a significant reduction in exposure to disease and potentially a reduction in the incidence of herpes zoster later in life [13–15]. Since the introduction of routine varicella-zoster vaccination in the United States of America (USA) in 1995, active surveillance of varicella in three communities has shown a decline of over 70% in reported cases and a significant decline in hospitalisation for varicella associated invasive Group A Streptococcal infection [16–19]. As the vaccine strain of varicella virus is expected to cause herpes zoster less frequently than wild type infection, not only varicella, but also herpes zoster could ultimately be eliminated once a high coverage rate is achieved [14].

Investigation of parental attitudes to varicella immunisation may help to explain why varicella vaccine uptake remains low despite inclusion in the ASVS. Although statistics indicate a growing awareness of the significant morbidity, mortality and escalating health care costs associated with complications of varicella disease, many parents choose not to have their children immunised [20]. Freeman et al. established that information about the vaccine and the recommendation of providers are important in determining a parent's decision about the vaccine for their child [6]. A study conducted in Hawaii (2001) demonstrated that lack of knowledge about the disease and the vaccine (25% of parents interviewed) was a significant factor in parents' decisions whether or not to have their child immunised [20]. However, studies examining parents' and providers' attitudes in the USA have less relevance to the Australian context as the vaccine is provided free for children covered by the Vaccines for Children Program in the USA. When considering a national vaccination program to increase coverage for a vaccine it is important to consider reasons why parents decide whether or not to immunise their children.

2. Methods

A cross sectional study was conducted using a telephone survey of randomly selected households in South Australia (SA). The survey was performed as part of the 'Health Monitor' program through the Population Research and Outcomes Studies Unit, Department of Health, in SA [21]. The random sample was based on the South Australian Electronic White Pages (EWP) telephone listings of households in SA, both city and rural. Only one interview per household was conducted. An adult, 18 years or older at the last birthday, was selected for an interview, and answered questions for *all the children* in the household aged less than 18 years. Interviews were conducted using the CATI (Computer Assisted Telephone Interviewing) methodology during which data obtained were entered from the interviewer's screen to the computer database. Three thousand, four hundred households were randomly selected from a total of 591,373 households in South Australia (Australian Bureau of Statistics (ABS), 2001 census). A pilot study of 50 randomly selected households was conducted in May 2004 to test question formats and sequence.

The survey was conducted to estimate the number of children 0–17 years of age who have been immunised with varicella vaccine from a random sample of children in metropolitan and rural SA. Information was then obtained to determine the main reasons why caregivers choose not to have their child immunised against varicella. Caregivers were asked whether their child had previously developed varicella infection and if they had received a varicella vaccine. Household contacts who responded "yes" to immunisation with varicella vaccine were then asked to provide the main reason why they had decided to have their child immunised. Household contacts who gave a "no" response to varicella immunisation were asked to provide the main reason why they had decided not to have their child immunised. These questions were posed as open-ended questions for each child in the household.

The survey data were weighted to the age, sex and geographical area profile of the population of South Australia and the probability of selection within a household. This methodology ensured that survey findings were applicable to the South Australian population as a whole. Individual data were weighted by the inverse of the individual's probability of selection and then reweighted to benchmarks derived from the ABS Estimated Resident Population for 30 June

2002 for SA. For questions regarding households rather than individuals, records were weighted by the inverse probability of the selection of the household then reweighted to benchmarks derived from the ABS 2001 Census of Population and Housing for occupied private dwellings by location [21].

The SEIFA (Socio Economic Index For Areas), 'Index of Relative Socioeconomic Disadvantage' was used as a measure of socio-economic status. The SEIFA index represents quartiles of socio-economic status by residential post-code based on income and educational attainment in the Australian population [22].

Statistical analyses were performed using the Stata computer package, using routines specifically designed to analyse clustered, weighted survey data [23]. Estimates of population percentages with 95% confidence intervals (95% CI) are presented.

The study protocol was reviewed and approved by the Women's and Children's Hospital Research Ethics Committee, Adelaide, South Australia.

3. Results

3.1. Health monitor survey

From 3400 telephone numbers selected, 621 could not be contacted or were not household numbers. From the remaining 2779 numbers, 2002 interviews were conducted in June 2004, with a participation rate of 72% (Table 1).

3.2. Description of study sample (raw data)

Six hundred and thirteen of the households interviewed contained children in the study age range of 0–17 years. These 613 households contained 1148 children, with a range of 1–7 children per household. In households interviewed, 21.2% (compared to 23.5% of children in South Australia, Australian Bureau of Statistics (ABS) 2004) were children aged 0–4 years ($n=242$), 32% (compared to 24.6% of children in South Australia, ABS 2004) were children aged 5–9 years ($n=366$), 31% (compared to 25.7% of children in South Australia, ABS 2004) were children aged 10–14 years of age ($n=355$) and 15.8% (compared to 26.2% of children in South Australia, ABS, 2004) were children aged 15–17 years of age ($n=181$). Age was not specified for four children. The study sample included 576 male and 572 female children.

Table 1
Household participation rate

Study population	Household participants
Initial Sample	3400
Reasons for sample loss	
Non residential numbers	95
Telstra message/disconnected	296
Fax/modem	17
Contact could not be established after ten calls	213
Remaining sample	2779
Non responders	
Refusal (not interested/too busy)	485
Unable to speak English	83
Illness/hearing impaired	98
Terminated interview	5
Respondent unavailable for duration of survey	106
Total interviews	2002
Households with children	613

Household demographic details were obtained. The median age of the household interviewee was 40.5 years with a range of 18–76 years compared to a median age of 38.5 years in the South Australia population. Contacts interviewed included 268 males (43.7% of the study population compared to 49% of the South Australian population, ABS 2004) and 345 females (56.3% of the study population compared to 51% of the South Australian population, ABS 2004). Sixty-nine percent ($n=423$) of households were situated in metropolitan Adelaide (compared to 73.3% of the South Australian population, ABS 2004) and 31% ($n=190$) were rural residences (compared to 26.7% of the South Australian population, ABS 2004).

3.3. Description of weighted data

Weighting was performed on the raw data collected from the 613 randomly selected households in the Health Monitor Survey. Including sampling weights in the analysis of the study population provides estimates that are unbiased in relation to the total population of SA. There was a near equal proportion of males (49%) and females (51%) within weighted households ($n=686$). The study results are based on a weighted survey sample of 636 males and 641 females ($n=1277$) between the ages of 0 and 17 years (Table 2). Almost 90% of caregivers interviewed in the weighted sample were the mother (44.4%), the father (40.3%), step parent (3.0%) or foster parent (0.5%) of the children in the household. Other household contacts included an older sibling (8.8%) or grandparent (2.1%) and the remaining 0.3% was classified as "other" contact.

3.4. Varicella infection

Almost 14% (95% CI 9.7, 19.6) of children in the youngest age group (0–4 years) had a history of clinically apparent varicella infection and by 15–18 years of age this had increased to 81.1% (95% CI 73.6, 86.8) (Table 3). These data are supported by the literature, which suggests 75% of children will have contracted varicella by the age of 12 years [3,24]. Caregivers were asked to report if the infection had been diagnosed by a doctor. Six hundred out of a total of 680 (88.2%) children were reported to have been reviewed by a doctor and varicella infection confirmed.

Table 2
Household demographics ($n = 686$ weighted data)

Household characteristics	Category	Number of respondents	Proportion of respondents (%)
Age of respondent (10-year intervals), $n = 686$	18 – 24 years	80	11.7
	25 – 34 years	159	23.2
	35 – 44 years	289	42.1
	45 – 54 years	142	20.7
	55 – 64 years	11	1.6
	>65 years	5	0.7
Gender, $n = 686$	Male	336	49.0
	Female	350	51.0
Socio-economic status Post-code (SEIFA index of disadvantage) measured in quartiles, $n = 684$	1st quartile (lowest socio-economic group)	191	27.9
	2nd quartile	152	22.2
	3rd quartile	139	20.3
	4th quartile (highest socio-economic group)	202	29.5
Highest educational qualification of interviewee, $n = 686$	Secondary school	312	45.5
	Trade	229	33.4
	Bachelor degree	145	21.1
Location of Residential Address, $n = 686$	Metropolitan	500	72.9
	Rural	186	27.1
Household Income, $n = 624$	0 – $20,000	31	5.0
	$20,000 – $60,000	275	44.1
	$60,000 – $80,000	148	23.7
	>$80,000	170	27.2
Country of birth, $n = 686$	Australia	562	81.9
	UK	68	9.9
	Other	56	8.2
Number of children in household, $n = 686$	1	280	40.8
	2	276	40.2
	3	91	13.3
	4	30	4.4
	5	4	0.6
	6	3	0.4
	7	2	0.3

Note: Proportions for each household characteristic may not add up to 100% due to rounding of figures to one decimal place.

Table 3
Varicella infection and varicella immunisation weighted to the population (95% CI for proportions)

Age groups	Varicella infection (%) (95% CI)	Varicella immunisation (%) (95% CI)	Varicella infection and immunisation (%) (95% CI)	No varicella infection, no immunisation (%) (95% CI)
Children 9 months to 4 years of age ($n = 294$)				
<9 months	0	0	0	32 (100%)
9 to <12 months	0	2 (22.7%) (5.5, 59.8)	0	8 (77.0%) (40.3, 94.5)
12 to <18 months	5 (15.9%) (5.9, 36.2)	9 (30.0%) (13.3, 54.5)	1 (4.2%) (0.6, 25.1)	17 (52.7%) (32.3, 72.3)
18 months to <2 years	0	10 (50.8%) (23.5, 77.6)	0	10 (40.7%) (19.2, 66.4)
2 to 4 years	35 (17.9%) (12.5, 25.0)	96 (51.8%) (42.6, 60.9)	8 (3.9%) (1.7, 8.8)	60 (30.5%) (22.9, 39.2)
Total (children 9 months to 4 years)[a]	40 (13.9%) (9.7, 19.6)	116 (48.0%) (39.4, 56.7)	9 (3.1%) (1.4, 6.5)	94 (31.9%) (25.1, 39.5)
Children 5 – 17 years of age				
5 – 9	226 (58.1%) (51.8, 64.1)	157 (44.8%) (37.8, 52.0)	66 (16.6%) (12.1, 22.3)	58 (14.6%) (10.7, 19.6)
10 – 14	254 (75.0%) (68.3, 80.5)	112 (36.7%) (30.1, 43.9)	62 (17.0%) (12.8, 22.3)	33 (9.1%) (5.4, 14.9)
15 – 18	160 (81.1%) (73.6, 86.8)	61 (37.1%) (28.7, 46.2)	33 (15.6%) (10.8, 22.0)	8 (3.8%) (1.9, 7.7)
Total (all children)	680 (56.1%) (51.8, 60.4)	446 (42.0%) (37.3, 46.8)	170 (13.4%) (10.9, 16.4)	193 (15.3%) (12.2, 19.0)

[a] Although the vaccine was previously recommended from 12 months of age and is currently recommended at 18 months of age, it is licensed from 9 months of age. ACIR and study data confirm that it is being administered from 9 months of age by some practitioners in Australia.

3.5. Varicella immunisation

Reporting a history of varicella immunisation decreased with increase in age of children (Table 3). Forty-eight percent (95% CI 39.4, 56.7) of children 9 months to 4 years of age had received varicella vaccine compared to 36.7% (95% CI 30.1, 43.9) of adolescents 10 – 14 years of age. The higher the educational qualification the less likely the caregiver was to have their child immunised against varicella infection (Wald test for coefficient on educational qualification from a survey weighted logistic regression model yields $p = 0.002$). Forty-eight percent of children (95% CI 40.8, 55.4) reported by a household contact who had completed secondary school were immunised compared to 29.2% of children (95% CI 21.6, 38.0) reported by a household contact who had obtained a bachelor degree or equivalent. This finding was significant for fathers ($\chi^2_{2df} = 61.289$, $p = 0.004$) reporting on history of immunisation but not for mothers ($\chi^2_{2df} = 12.827$, $p = 0.178$). However, there was no association found between work status of the interviewee, socio-economic status or household income and uptake of varicella vaccine. No association was identified between administration of the vaccine and the child's gender or residential address, suggesting the vaccine is readily accessible state-wide. Fourteen percent of responders did not know or could not remember whether or not their child had received a varicella vaccine. A higher proportion of responders from the lowest socio-economic quartile reported a "do not know" response. However, there were no other differences in characteristics of this group compared to the study population.

Almost 53% (95% CI 32.3, 72.3) of children aged 12 to <18 months of age and 41% (95% CI 19.2, 66.4) of children aged 18 to <24 months were at risk of varicella infection as they had no history of prior infection and had not been immunised. The proportion of children at risk decreased with age as there were a higher proportion of children with wild type varicella immunity amongst older children.

3.6. Reasons why caregivers chose not to have their child immunised with varicella vaccine

Caregivers who answered "no" to their child receiving varicella vaccine were asked "what was your main reason for not immunising (him/her) against chicken pox infection?" as an open-ended question. Although caregivers were asked to provide a single response, 8.0% of responders were only able to provide multiple responses. As respondents giving multiple responses did not fulfil the study criteria of providing the main reason for not immunising their child, they were excluded from this analysis. There were no statistically significant differences in gender, age or socio-economic status between the groups that provided single or multiple responses. The main reason reported was previous varicella infection (Table 4). If children with previous infection were excluded, 20.1% of the remaining responses included a lack of knowledge that a vaccine is available to prevent varicella. A similar proportion, 19.2%, reported they did not have their child immunised because the vaccine is not included on the childhood immunisation schedule. In combination, these two categories accounted for nearly 40% of the responses. The third most common reason provided was the cost of the vaccine (14.7%). Immunisation had been delayed but was planned for 11.8% of these children and 10.5% reported unavailability of the vaccine when their child received routine childhood immunisations. Only 4.2% deferred immunisation due to concern about side effects of the vaccine.

There was no significant association found between age or relationship of interviewee, highest educational qualification obtained or household income for the main reasons provided for failure to immunise a child. A significant association was found ($\chi^2_{3df} = 23.538$, $p = 0.049$) between cost

Table 4
Reasons why children were not immunised with varicella vaccine (weighted to the population)

Main reasons why children are not immunised with varicella vaccine ($n = 513$)	Number of responses	95% CI	Proportion of all responses (%)	95% CI
Previous chicken pox infection	177	(143.6, 210.7)	34.5	(28.7, 41.3)
Unaware chicken pox vaccine available	63	(36.4, 88.9)	12.3	(8.2, 18.1)
Chicken pox vaccine not included on the childhood schedule	60	(37.4, 82.7)	11.7	(8.0, 16.9)
Cost	46	(19.0, 72.1)	8.9	(5.1,15.3)
Planned but delayed immunisation against chicken pox	37	(19.0, 51.9)	7.2	(4.4, 10.9)
Vaccine unavailable at time childhood vaccinations given	33	(17.9, 47.1)	6.4	(4.0, 9.9)
Child reported to be too young to receive vaccine	23	(10.3, 35.0)	4.5	(2.5, 7.6)
Concern about side effects following immunisation	12	(2.6, 20.4)	2.3	(1.0, 4.9)
Anti-immunisation (in general)	6	(0.8, 11.6)	1.2	(0.5, 2.9)
Chicken pox infection considered a mild disease/no concern	6	(0, 12.4)	1.2	(0.5, 2.9)
Preferred child developed immunity from natural infection	5	(0, 11.6)	1.0	(0.3, 3.3)
Partner's responsibility	4	(0, 11.9)	0.8	(0.1, 5.4)
Chicken pox vaccine not offered	3	(0, 7.1)	0.6	(0.2, 2.0)
Chicken pox vaccine is ineffective	2	(0, 5.0)	0.4	(0.1, 1.6)
Vaccine may cause chicken pox infection	1	(0, 3.0)	0.2	(0, 1.4)
No reason	15	(3.4, 25.6)	2.9	(1.3, 6.0)
Do not know/cannot remember	20	(7.9, 30.0)	3.9	(2.1, 6.6)

Table 5
Reasons why children were immunised with varicella vaccine (weighted to the population)

Main reasons why children were immunised with varicella vaccine ($n=408$)	Number of responses	95% CI	Proportion of all responses (%)	95% CI
Concern about acquiring varicella infection	268	(225.1, 310.0)	65.7	(58.3, 72.3)
Recommended on vaccination schedule	44	(27.8, 60.6)	10.8	(7.4, 15.7)
Doctor recommended the vaccine	25	(12.6, 38.2)	6.1	(3.8, 10.2)
Pro-immunisation in general	19	(8.4, 29.0)	4.7	(2.6, 8.0)
School/childcare recommended vaccine	6	(0, 13.6)	1.5	(0.3, 5.6)
Concern about transmitting infection to others	4	(0, 11.4)	1.0	(0.2, 5.5)
Contact with an infected child	3	(0, 5.7)	0.7	(0.1, 2.5)
Other	11	(1.2, 18.5)	2.7	(0.8, 4.2)
No reason	8	(0.1, 15.0)	2.0	(0.7, 4.9)
Cannot recall/do not know	20	(3.1, 32.2)	4.9	(1.9, 9.6)

of the vaccine as the main reason for not immunising a child and socio-economic status. Only 4.7% (95% CI 1.6, 12.9) of respondents in the highest socio-economic quartile compared to 15.3% (95% CI 7.6, 28.5) in the second quartile (low to middle socio-economic status) cited cost as the reason for not complying with recommendations. There was also a significant association ($\chi^2_{6df}=54.240$, $p=0.043$) between cost and the number of children in the family. Only 4.0% (95% CI 1.5, 11.2) of interviewees with one child nominated cost as the main concern compared to 13.6% (95% CI 6.0, 27.8) of families with three children and 52.0% (95% CI 8.7, 92.5) of families with five children.

3.7. Reasons why caregivers chose to have their child immunised with varicella vaccine

Caregivers who responded "yes" to previous varicella immunisation were asked to provide the main reason for this decision. Eight percent of household contacts were unable to give a single response and were excluded from further analysis. The majority of caregivers (65.7%) had their children immunised with varicella vaccine to prevent varicella infection (Table 5). Over 10% of caregivers chose to immunise their children because the vaccine was included on the ASVS. Schools and child care centres were active in recommending the vaccine to parents. There was no significant association between recognition that varicella vaccine is included on the ASVS and socio-economic status, relationship of interviewee to child or highest educational qualification achieved by the interviewee. However, a significant association ($\chi^2_{3df}=19.980$, $p=0.008$) was found between parental concern about a child acquiring the infection and household income. Twenty-eight percent (95% CI 11.1, 56.3) of responders in the lowest income group (<A$20,000) gave prevention of disease as the main reason for immunising their child compared to 75.8% (95% CI 64.9, 84.1) in the middle income group (A$20,000 – $60,000) and 53.0% (95% CI 39.1, 67.1) of interviewees in the high income group (>A$80,000). There was no association found between gender of the interviewee, highest educational qualification achieved or socio-economic status and concern about acquiring varicella infection.

Average annual uptake of varicella vaccine in South Australia (since 2000) was estimated to be 11.3% in children aged 9 months to 4 years of age, 10.5% in children 5 – 10 years of age, 8.6% in children 11 – 14 years of age and 8.7% in adolescents 15 – 18 years of age.

4. Discussion

The results of this study justify concern that there is inadequate varicella vaccine coverage in children in South Australia. This is particularly evident in the 9 month to 4 years age group who have been targeted for immunisation and who experience the highest rates of hospitalisation for varicella infection [1]. Only 30% of infants 12 – 18 months of age, 50.8% of infants aged 18 months to 2 years and 51.8% of 2 – 4-year olds have received a varicella vaccine, which is consistent with low to moderate coverage in the 2 – 4-year age group. Over 50% (52.1%) of susceptible children less than 5 years of age have received a varicella vaccine compared to 67.3% of susceptible children aged 5 – 9 years of age and 78.8% of susceptible children 10 – 14 years of age. As the risk of acquiring disease increases with age, the proportion of susceptible children eligible for varicella vaccine decreases (Table 3).

The estimated annual vaccine coverage in children 9 months to 4 years of age is higher than the uptake calculated from vaccine sales in 2003. The difference observed might be due to recall bias although the data obtained in the study population are more recent than the vaccine sales data provided by GSK. This may also represent an increase in immunisation since introduction of the vaccine onto the ASVS in September 2003, as suggested by data from the ACIR. The Commonwealth Serum Laboratories (CSL), the distributor of VARIVAX® (Merck & Co), have reported a 149% increase in sales between 2003 and 2004 which is consistent with our results.

The proportion of children in the study with a history of varicella infection is consistent with previous estimates [3,24]. A large majority of caregivers (88.2%) considered the infection significant enough to visit a doctor, which coun-

ters the claim that parents consider chicken pox to be a mild disease of little concern. Data on history of varicella may be subject to recall bias. However, previous studies have shown that a history of chicken pox infection from a parent is a reliable measure of immunity because the rash from varicella is so distinctive and sub clinical cases are unusual [3,25].

A substantial proportion of children (up to 17% depending on age) were reported as having had both varicella infection and varicella vaccine administered. Several explanations are possible. There was a significant association ($\chi^2_{3df} = 32.049$, $p = 0.0005$) between age of the child and a history of both infection and vaccination, which is most likely due to the increase in risk of acquiring varicella infection with increasing age. Parental recall may not be as accurate for older children as it is for younger children. Three household contacts reported the main reason for their child receiving varicella vaccine was "so she would not be so sick if she got it (varicella infection) again" or "worried about getting it (varicella infection) a second time". However, another possible explanation for a history of infection and vaccination is a moderately high proportion of breakthrough cases of varicella infection following immunisation with the vaccine. Overall, seroconversion occurs in 90–100% of those vaccinated and about 70–90% are protected when exposed subsequently to infection within the household. Breakthrough infection after exposure occurs at a rate of 1–3% a year in those vaccinated, although these cases are usually mild [26–28]. In a recent assessment of a varicella outbreak in a school in Oregon in the USA, the estimate of cases occurring in children who had been vaccinated was 12% [29]. A previous study reported the risk of breakthrough varicella 5–10 years after immunisation was 18.6% [30]. In our cross-sectional study, unfortunately we were unable to determine whether immunisation preceded the infection (because of limitations in the questionnaire design).

There are no known published Australian data on reasons why parents choose whether or not to immunise their children against varicella infection. Studies elsewhere have shown that in general, demographic background variables do not affect parents' perceptions about the vaccine [20]. Studies have been conducted in the USA, where uptake of the vaccine has been high, so a direct comparison is unreliable. In the study conducted in Hawaii, USA, 71% of the children in the sample had received the varicella vaccine [20]. The majority of participants were not concerned about side effects from the vaccine but were concerned about immunity waning over time. Almost all the participants (96%) thought their child would require a booster dose of varicella vaccine. The main reasons reported in our study for not having children immunised with varicella vaccine were related to lack of funding and knowledge about the vaccine rather than concerns about the vaccine or associated side effects. The three most commonly cited reasons (excluding previous varicella infection) for not immunising a child were due to lack of knowledge about the vaccine, lack of awareness that the vaccine was included on the ASVS, and the cost of the vaccine. Most caregivers identified prevention of disease as the primary reason for immunising their child. Doctors have an important role in advising and educating parents about the vaccine. Six percent of interviewees reported that the main reason why their children were immunised was due to a doctor's recommendation. This response was particularly evident in the lower socio-economic group.

The strength of this study is the large number of children randomly sampled from the state of SA and weighted to the population to improve the generalisability of the data. This is a cross-sectional study and as such has limitations in time measures including changing parental opinions. A caregiver's response provided during the survey may be different from the original reason discussed at the time the child was eligible for the immunisation. The telephone survey only allowed inclusion of English speaking households due to the impracticality of providing interpreters. As non-English speaking households represent a group at risk of low immunisation coverage, this group should be assessed using different methodology.

Studies of vaccination history are subject to recall bias. A limitation of this sampling method is that the primary care giver is not identified. Data provided by the primary care giver may be less subject to recall bias. A history of varicella immunisation status was compared between mother, father and all interviewees as responders and an annual varicella vaccine up-take calculated. The highest uptake reported by all household contacts occurred in the 9-month to 4-year-old age group (11.3% annually) which was consistent with ACIR data and reported consistently by both parents. A significant difference in responses was identified in households where the mother was a respondent (7.0% annually) compared to households in which the father was a respondent (13.7% annually) for children 15–18 years of age. This was also seen for children 5–14 years of age. This observed difference suggests recall bias in the data for older children but not for young children. These data demonstrate a higher varicella uptake than data previously reported on the ACIR and by GSK. The overall reported percentage of children with a history of infection was consistent with previous studies [1,3]. We did not attempt to confirm cases of varicella infection and previous immunisation by examining medical or immunisation records due to the large sample size and privacy concerns. Households randomly selected from listed telephone numbers may lead to bias as households without a land-line telephone or whose telephone numbers are not listed are excluded from the population sample. In South Australia it is estimated that 3.2% of households do not have a telephone. The indigenous population, lower income households and the unemployed who experience a high burden of disease are overrepresented in this group and therefore underrepresented in this study.

The number of responders who reported themselves as being "anti-immunisation in general" was relatively low (1.2%) and is consistent with previous literature [31].

5. Conclusion

There is evidence that despite varicella vaccine being recommended on the ASVS for almost a year, uptake of the vaccine remains low in South Australia, and probably in Australia as a whole. Introduction of new vaccines to the ASVS requires surveillance of both the uptake of the vaccine in addition to careful surveillance of infection. In particular, routine varicella immunization has the potential to change the epidemiology of the infection and close surveillance of both uptake and disease is required. Administration of all immunizations that are recommended and included on the ASVS (whether or not they are funded) should be recorded on the ACIR. Accurate vaccine coverage data are essential in understanding the epidemiological impact of vaccine programs.

Barriers to varicella immunisation are the result of poor knowledge about the vaccine and lack of funding. As the vaccine is recommended but not yet funded, the vaccine is incorrectly assessed by many parents as not being part of the ASVS. Recommending the vaccine but not providing funding gives "mixed messages" to immunisation providers and to parents and caregivers. The number of parents who were unaware that a varicella vaccine is available is of concern and was reported equally by mothers and fathers. The study results reported here suggest that parents are not well informed about the vaccine and parental education will need to be a significant component of an improved immunisation campaign to increase varicella coverage. However, the most important consideration is likely to be provision of Governmental funding for varicella vaccine. Our study data suggest that once funding is provided a high coverage rate for varicella vaccine can be achieved and a ubiquitous infectious disease may be eliminated.

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ORIGINAL ARTICLE

Varicella immunisation practice: Implications for provision of a recommended, non-funded vaccine

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Aim: In Australia in 2003 a two-tiered immunisation schedule was introduced consisting of funded (National Immunisation Program) and non-funded but recommended vaccines (Best Practice Schedule), including varicella vaccine. The aim of this study was to examine immunisation practice when a vaccine is recommended but not funded by Government.

Methods: A survey was sent to 600 randomly selected general practitioners (GPs) in South Australia between June and August 2005, prior to provision of Federal funding for varicella vaccine.

Results: Although varicella was considered an important disease to prevent by 89% of GPs, only 25% of GPs always discussed the non-funded immunisation with parents at the time of a routine immunisation visit. Female GPs were more likely to discuss immunisation with recommended, non-funded vaccines than male GPs. Those who were supportive of varicella prevention were more likely to discuss immunisation with the non-funded vaccine. GPs who always provided information about the disease were more likely to have parents accept their advice about varicella vaccine (62.7%) than those who never provided information (40%). GPs reported parental refusal of varicella vaccine was due to the cost and perception that varicella is a mild disease.

Conclusions: The results of this study showed variability in prescribing practices for a non-funded vaccine. Recommending a vaccine without provision of funding may lead to 'mixed messages' for immunisation providers and parents with resultant low coverage. Funding a vaccine is likely to reduce variability in provision of the vaccine and improve coverage in the community.

Key words: education; general paediatrics; immunisation; infectious disease.

Although generally varicella is a mild infection, there are approximately 1500 hospitalisations and 10–20 deaths from varicella or related complications each year in Australia.[1,2] To immunocompromised individuals up to 36% develop disseminated disease.[3] The cost of varicella to the community is considerable not only for medical opinion and hospitalisations but for time taken off work by parents to look after infected children and subsequent infection in siblings and occasionally parents.[4,5]

Key Points

1. Recommending a vaccine without providing funding is likely to result in low coverage.
2. Funding-recommended vaccines improves equity of access and reduces variability in approach to provision and promotion of the vaccine in the community.
3. Provision of information about the preventable disease and specific information about the vaccine is more likely to result in acceptance of the immunisation by the parent as reported by GPs.

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In Australia, despite recent provision of funding for varicella immunisation at 18 months of age, varicella vaccine uptake has been slower than expected, resulting in incomplete coverage.[4,6] A partially immunised community is of concern because of the possibility of induced changes in epidemiology of varicella disease.[7] If a proportion of the population is immunised, there is less contact with wild-type varicella in childhood with a higher risk of developing the disease at an older age when the disease is more serious and more costly to the community.[5,8–11] If varicella vaccine immunisation rates remain low, the number of children who become susceptible adults will increase, and these susceptible adults will be more likely to contract varicella from the cohort of unimmunised children.

Accurate estimates of varicella vaccination coverage in Australia are not available as recording of varicella vaccination on the Australian Childhood Immunisation Register (ACIR) is not yet linked to immunisation provider incentive payments. A high coverage rate in the population (at least 75%) has many benefits, including a significant reduction in exposure to disease and potentially a reduction in the incidence of herpes zoster later in life.[12–14] Modelling has also suggested the possibility of a

temporary increase in varicella at an older age with high coverage in children, although this has not been demonstrated with expanded use of the vaccine.[7]

Although varicella vaccine was approved for use and recommended by the National Health and Medical Research Council for children from 12 months of age in 2000, it was not until 1 November 2005 that Federal funding for provision of the vaccine at 18 months of age was established.[15,16] In March 2005 the Minister for Health announced that Commonwealth funding for varicella vaccine at 18 months of age with a catch-up vaccination (no previous varicella) for children between 10 and 13 years would be provided under the National Immunisation Program in response to recommendations made in January 2005 by the Technical Advisory Group on Immunisation.

The survey, which forms the basis of this report, was conducted in June 2005, prior to provision of Federal funding for varicella vaccine in the National Immunisation Program, and represents an example of general practitioner (GP) use of a vaccine that is recommended but not funded by government. This survey assessed GP attitudes to and variability in prescribing a recommended, non-funded vaccine.

Methods

General practitioners were randomly selected from the Australian Medical Publishing Company (AMPCo) database. This database contains a near complete list (98.4%) of registered and practising GPs in metropolitan and rural South Australia (2003). As our survey sought answers to several questions, we based our sample size on the proportion to be estimated with the highest variance (i.e. 0.5), which leads to a conservative (i.e. larger) sample size. To estimate such a proportion with sampling error of 0.05, and allowing for a finite population correction, required approximately 300 GPs to be surveyed. Assuming a questionnaire return rate of 50%, the questionnaire was sent to 600 randomly selected GPs.

A pilot study of 50 GPs was conducted in April 2005 to test question formats and sequence. GPs were asked to provide written comment on the questionnaire, but no concerns or queries were raised by GPs involved in the pilot study. The questionnaire was initially mailed in June 2005, with a follow-up questionnaire sent to GPs who had not responded by 6 weeks post mail-out.

The survey questions were aligned to key messages about the use of non-funded recommended vaccines using varicella vaccine as an example. The questionnaire was designed in a brief double-sided, one-page format with 14 immunisation questions and six demographic questions. Demographic characteristics included GP gender, year of graduation, practice postcode (the name of the practitioner was not recorded to ensure anonymity of responses), average number of appointments per week, number of childhood immunisations completed each week and number of varicella immunisations administered per month. Information was obtained to assess factors that may contribute to prescription of a recommended but non-funded vaccine, including concerns about severity of disease or adverse events related to the vaccine. Questions relating to number of sessions or number of immunisations were answered by selection of ordinal categories numbered 1–5. The Likert scale (five categories) was used to assess GP responses to questions about disease severity, while provision of information to parents was assessed using a four-category scale: always, frequently, sometimes, never. Statistical analyses were performed using the Stata computer package.[17] Statistical tests were two-tailed using a significance level of 5%. Associations between variables were sought using a χ^2-test.

The study protocol was approved by the Children, Youth and Women's Health Service Research Ethics Committee, Adelaide, South Australia, and the study was conducted in accordance with the Declaration of Helsinki.

Results

GP survey population

A return rate of 53% with 90% evaluable responses was accomplished with the two mail-outs to GP practices (Fig. 1). The responses from 285 GPs from metropolitan and rural South Australia are presented.

There were 169 (59.7%) male GPs who completed the survey and 108 (38.2%) female GPs (2.1% gender not provided). In Australia, 65% of practising primary care physicians are male and 35% are female.[18] The number of years since graduation ranged from 6 to 59 years with a mean of 23.9 years (95% CI 22.8–25.1) and a median of 21 years (95% CI 19–23) (Table 1). There was a difference in gender for years since graduation with a mean of 24 years (95% CI 22.5–25.8) for male GPs compared with 18.5 years (95% CI 16.8–20.5) for female GPs. The highest proportion of practitioners (42.8%) was from practices seeing at least 120 patients per week. The return sample of GPs was representative of GPs from both metropolitan and rural divisions with similar proportions of GPs from each division in the returned and non-returned groups (Fig. 1).

Two hundred and eight (75.6%) GPs practised in metropolitan Adelaide and 67 (24.4%) practised in rural South Australia. In Australia, 73% of GPs work in metropolitan practices and 27% in rural locations.[16]

Administration of varicella vaccine

Seventy per cent of GPs reported administration of at least one varicella vaccine per month. The higher the number of paediatric consultations performed, the greater the number of varicella vaccine doses administered ($\chi^2_{5\text{d.f.}} = 45.076$, $P < 0.001$) (Fig. 2). GPs who had recently graduated (≤20 years since graduation) were more likely to provide varicella vaccine, with a decrease in proportion of GPs administering the vaccine as years since graduation increased (Fig. 3). The score test for the trend in odds of giving varicella versus no varicella with increasing number of years since graduation yielded $\chi^2_{1\text{d.f.}} = 4.16$, $P < 0.041$. The decreasing trend in vaccination with increasing years since graduation persisted when adjusted for the number of paediatric consultations per week. GPs who gave fewer routine childhood immunisations per week were less likely to administer varicella vaccine ($\chi^2_{3\text{d.f.}} = 64.453$, $P < 0.001$).

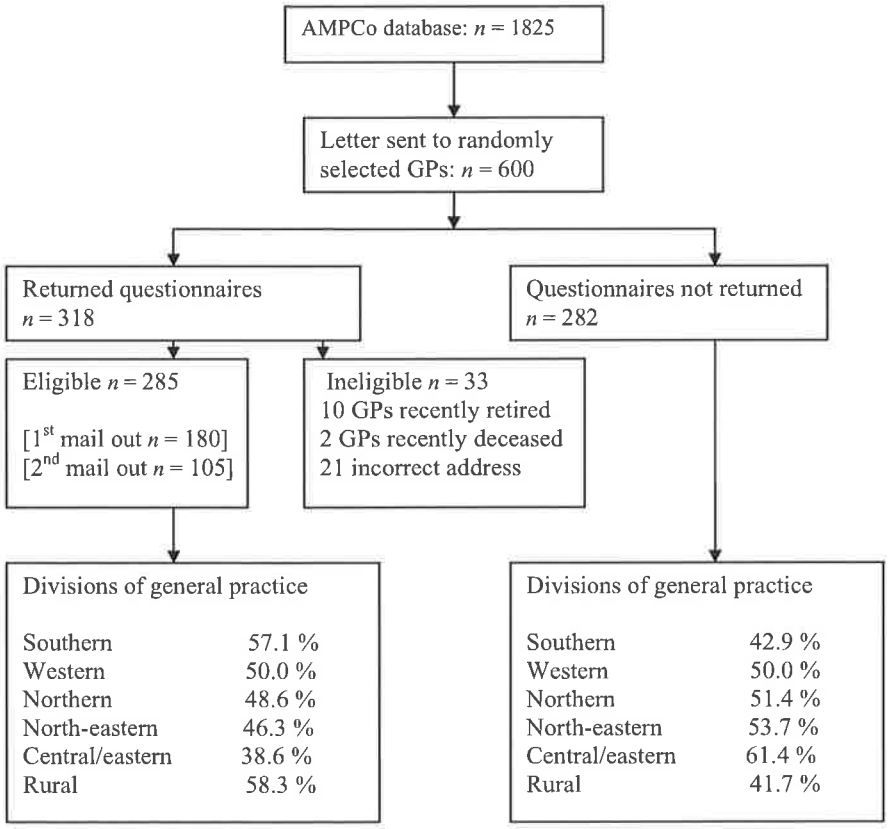

Fig. 1 Study profile including distribution of responses from metropolitan and rural South Australia. AMPCo database, Australian Medical Publishing Company database; GP, general practitioner.

Table 1 GP demographics

GP demographics	Category	Number of respondents	Proportion of respondents (%)
Years since graduation (10-year intervals) (*n* = 277), years	1–10	23	8.3
	11–20	90	32.5
	21–30	84	30.3
	31–40	64	23.1
	41–50	14	5.1
	51–60	2	0.7
Gender (*n* = 277)	Male	169	61.1
	Female	108	38.9
Location of GP practice (*n* = 275)	Metropolitan	208	75.6
	Rural		24.4
	Large rural centre	2	0.7
	Small rural centre	17	6.2
	Other rural area	47	17.1
	Remote	1	0.4

Proportions for each GP characteristic may not add up to 100% because of rounding of figures to one decimal place. GP, general practitioner.

Prevention of infection by vaccination

Varicella was considered an important disease to prevent by 89.3% of GPs, with a small but not significant difference detected between male (87%) and female (93.5%) GPs. A gender difference was identified in recommending non-funded vaccines, such as varicella vaccine to patients at a routine immunisation visit ($\chi^2_{3d.f.}$ = 8.7863, P = 0.032). Twenty per cent of male GPs compared with 34.6% of female GPs always discussed immunisation with recommended but non-funded vaccines at a routine immunisation visit.

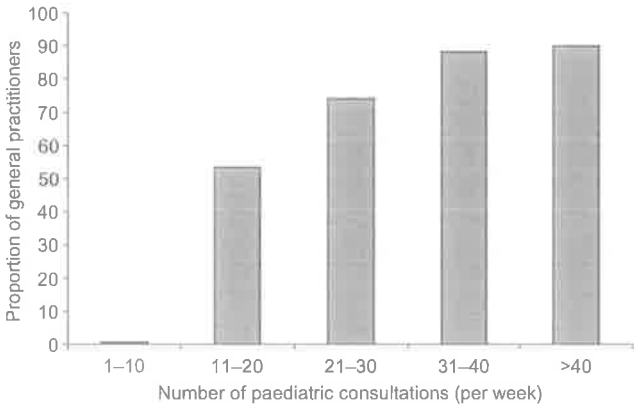

Fig. 2 Proportion of GPs who administer varicella vaccine (per month) by number of paediatric consultations per week. GP, general practitioner.

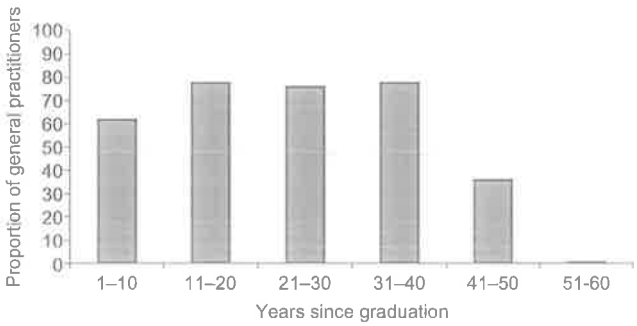

Fig. 3 Proportion of GPs who administer varicella vaccine (per month) by years since graduation. GP, general practitioner.

A minority of GPs (9.2%) identified concerns about adverse events related to varicella vaccine, including long-term effectiveness and safety of the vaccine and the potential for development of herpes zoster.

Parental acceptance of advice for immunisation with a recommended, non-funded vaccine

A minority of GPs (2.2% (n = 6)) agreed that patients always accept their advice, 50.5% (n = 140) agreed that parents frequently accepted their advice and 45.9% (n = 127) and 1.4%, respectively, agreed they sometimes or never accepted their advice. Recent graduates reported more success with parental acceptance of advice (60.7%) than less recent graduates (48.4%) ($\chi^2_{3d.f.}$ = 14.630, P = 0.002).

Twenty-eight per cent of GPs who considered varicella an important disease to prevent always discussed immunisation with non-funded vaccines at a routine immunisation visit, compared with 10% of GPs who were not concerned about the disease. GPs who considered varicella an important disease to prevent were more likely to discuss immunisation with recommended but non-funded vaccines ($\chi^2_{3d.f.}$ = 8.612, P = 0.035) and to provide parents with information about the disease ($\chi^2_{3d.f.}$ = 9.161, P = 0.027).

Table 2 Reasons for parents to decline varicella immunisation as reported by GPs

Category (n = 278)	Number and proportion of GPs who identified reason for parents to decline varicella immunisation	
	n	%
Cost	196	70.5
Varicella considered a mild disease	46	16.5
Already too many vaccines/injections	20	7.2
Prefer natural immunity	15	5.4
Concern about side effects of the vaccine	11	4.0
Varicella prevention considered a low priority	9	3.2
Not routine/not funded	5	1.8
Previous varicella infection	5	1.8
Concern about long-term effectiveness	3	1.1
Other	8	2.9

Total number (n = 318) and proportion >100% as multiple reasons were provided by some GPs. GP, general practitioner.

Provision of information to parents about non-funded, recommended vaccines and vaccine preventable diseases

Twenty-four per cent of GPs indicated they always provided detailed information about the preventable disease, 36.7 % frequently provided information, 31.3% sometimes provided information and 7.6% never provided information. Among GPs who considered varicella an important disease to prevent, 26.8% always provided information compared with 6.7% of GPs who were not concerned. GPs who always provided information about the preventable disease were more likely to report parents accepting their advice about vaccination with varicella vaccine (62.7%) than those who never provided information (40.0%) about the disease (P = 0.065).

Forty per cent of GPs always provided specific information to parents about the vaccine/s they are about to administer and 2.9% never provided specific information about the vaccine/s. GPs who always provided information were more likely to report parents accepting their advice about varicella vaccination (χ^2_{3d} = 7.8299, P = 0.050); 60.9% of parents compared with 28.6% whose GP never provided information.

According to the GPs surveyed, the most common reasons for parents to decline varicella immunisation were cost and the perception that varicella is a mild disease (Table 2).

Australian Childhood Immunisation Register notification

Over 70% of GPs surveyed (71.2%; n = 193) always reported varicella vaccination to the ACIR, a further 10.7% (n = 29)

reported varicella vaccination frequently, 5.5% (n = 15) reported sometimes and 12.6% (n = 34) never reported varicella vaccination. Female GPs were more likely to report varicella vaccination to the ACIR (77.7%) than male GPs (67.3%) ($\chi^2_{3d.f.}$ = 7.954, P = 0.047).

Univariate analysis identified factors significantly associated with GPs' provision of varicella vaccine. These included GPs' concerns about severity of disease, number of paediatric consultations, number of immunisations given per month and years since graduation. Multivariate analysis confirmed that the number of paediatric consultations per month and gender (OR = 1.73; 95% CI 1.37–2.18; P < 0.001) were predictors of whether or not a GP prescribed varicella vaccine (OR = 1.99; 95% CI 1.11–3.56; P = 0.021), where female GPs and increasing number of paediatric consultations resulted in increased provision of varicella. When the model was adjusted for number of paediatric consultations, gender became an important predictor of varicella use.

Discussion

Our survey of 285 randomly selected South Australian GPs found that there is variability in GP prescribing practices for non-funded vaccines such as varicella vaccine.

Although the number of paediatric consultations by a GP appeared to be strongly associated with provision of a non-funded vaccine to children at routine immunisation visits, other factors were important in acceptance by parents, including provision of information about the vaccine and the preventable disease.

The difference in vaccine use demonstrated for GPs' paediatric experience and years since graduation is possibly due to recent graduates having received more intensive teaching and training on immunisation, including the newer vaccines as an important aspect of preventive health care. Female GPs were more likely to discuss non-funded vaccines with their patients. This may have been due to the younger age of female GPs returning questionnaires or possibly due to longer time spent in consultation with a patient compared with consultation time with a male GP.[18,19] Female GPs were also more likely to record varicella immunisation on the ACIR, suggesting that female GPs may be more conscientious and therefore more likely to discuss non-funded vaccines with their patients.

Discussion about non-funded vaccines requires additional time and assessment and may be given less priority than discussion about funded vaccines by GPs because of less emphasis from state immunisation authorities. Recommending a vaccine without providing funding may result in variability in recommendations to parents from GPs and GP judgement of parental affordability of the vaccine. Our study suggests that when parents are provided with information about the vaccine, they are more likely to accept their GP's recommendation as reported by GPs. Provision of information about the vaccine is likely to instil confidence in parents that an informed decision has been made to have their child immunised. Provision of a fact sheet summarising information about the vaccine preventable disease and the vaccine at the time of patient booking could improve the decision-making process for the parent without impacting significantly on consultation time'.[20]

Reasons cited by GPs for parents not accepting varicella immunisation, including cost and the perception that varicella is a mild disease, were similar to reasons cited by household contacts in a community survey conducted in 2003.[4]

GPs surveyed identified reasons they had not prescribed varicella vaccine. Although the majority of these reasons were justified, reasons reported, such as the child having an egg allergy or the child's mother being pregnant, are not contraindications. Provision of brief and accurate information about new vaccines to GPs who are time pressured is likely to improve recommendation of the vaccine.

Attempts to estimate varicella vaccine coverage prior to funding of the vaccine have suggested low coverage, and more recent data suggest coverage in Australia remains inadequate since funding was introduced (74.8 % coverage for 2-year-olds in Australia, ACIR March 2007).[4] Recent estimates show variability in coverage between states, ranging from 70.2% in Western Australia to 80.1% in the Northern Territory (ACIR March 2007). Delays linking varicella vaccination to the ACIR and GP incentive payments suggest varicella uptake data are underestimated. Coverage data and uptake of new vaccines that are not funded under the NIP are not available.

Once incentive linked notification for varicella vaccine is established, accurate coverage rates can be determined. However, our study results suggest that the estimated coverage figures are likely to be reasonably accurate with over 70% of GPs advising that they always report varicella vaccination to the ACIR and only 12.6% advising that they never report non-funded vaccines to the ACIR. Accurate data on vaccine coverage are essential, particularly for varicella vaccine where partial coverage may lead to less exposure to infection during childhood with increased susceptibility and severity of infection at an older age.

The GP educational campaigns to improve coverage should include information about disease severity and complications, including the risk of inducing change in the epidemiology of the disease in the absence of high vaccine coverage. Although the majority of GPs identified appropriate reasons for advising against use of varicella vaccine, other reasons offered would not be considered as contraindications, for example, a mother who is currently pregnant, or prior clinical infection in siblings. Concerns raised by GPs were similar to concerns raised in other studies conducted in the USA, where variability in varicella funding exists.[21–24] In a study conducted in Canada, when varicella vaccine was recommended but not funded, physician recommendation was a strong determinant of vaccine uptake.[25]

Recent changes to the recommendations for varicella immunisation include receipt of two doses of varicella-containing vaccine to provide increased protection and minimise break through disease in children.[26] However, routine administration of a second dose of varicella-containing vaccine is not included on the National Immunisation Program and was rejected by the Pharmaceutical Benefits Advisory Committee following recommendation by the Australian Technical Advisory Committee because of lack of evidence of cost-effectiveness of a second dose. Education of GPs about recommendations to improve protection of children is of paramount importance, particularly when funding of an optimal vaccination program is not guaranteed.

Our sample reflected the gender and metropolitan/regional distribution of GPs in Australia. A response rate of approximately 50% may lead to bias in the study results. However, all general practice area divisions were well represented in the study population with slightly fewer GPs from the Eastern and Central Division in the responder group. The conclusions drawn from the study in relation to vaccine uptake by parents are limited, as no data were obtained on the demographic or socio-economic status of the patients. Although socio-economic status was not assessed, practice locations from each division were equally represented (Fig. 1).

A follow-up study would be instructive to compare GP attitudes and uptake of the vaccine since varicella vaccine became funded by the Federal Government. Qualitative data on how GPs make decisions about whether to offer a recommended non-funded vaccine would also be of value.

Our findings regarding acceptance and promotion of recommended, non-funded vaccines have implications for the introduction of new vaccines. When a new vaccine such as varicella vaccine becomes available, its use may be delayed because of limited information or lack of immunisation provider knowledge and experience. New vaccines for prevention of herpes zoster are in development with one recently licensed for use in Australia in adults of ≥60 years of age. Funding for this vaccine is not assured and is currently being negotiated. The findings of this study suggest that there will be variability in prescribing the vaccine and inequality in access if funding is not provided.

Although now funded for select age groups, the recently licensed Human Papillomavirus and rotavirus vaccines were initially only available for purchase. Judgement based on a GP's or parent's perception of severity of disease or a family's financial status may determine whether discussion about use of non-funded vaccines takes place at a routine immunisation visit.[4] Inequality in adoption of preventative health-care measures may result, where the wealthy are better protected from infectious diseases. Funding a recommended vaccine improves equity of access and reduces variability in approach to provision and promotion of the vaccine in the community. When a vaccine is introduced, provision of adequate and accurate information about the disease to be prevented, in addition to information about the vaccine, is likely to enhance acceptance of the vaccine by both GPs and parents.[23] Clear and consistent information coupled with support from Government to provide equal access to vaccines is essential to achieve adequate coverage in the community.

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This manuscript is dedicated to the memory of Dr Jack Marshall AM, a pioneer in Medical Education for General Practitioners.

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A cross-sectional survey to assess community attitudes to introduction of Human Papillomavirus vaccine

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Abstract

Objective: A vaccine to prevent human papilloma virus (HPV) infection has been licensed recently in the United States of America and Australia. The aim of this study was to assess community attitudes to the introduction of HPV vaccine in the State of South Australia.

Methods: A cross-sectional survey was conducted by computer-aided telephone interviews in February 2006. The survey assessed adult and parental attitudes to the introduction of HPV vaccine to provide protection against a sexually transmitted disease caused by HPV and against cervical cancer. Two thousand interviews were conducted in metropolitan and rural households.

Results: Two per cent of respondents knew that persistent HPV infection caused cervical cancer and a further 7% were aware that the cause was viral. The majority of adults interviewed (83%) considered that both men and women should receive HPV vaccine and 77% of parents agreed that they would have their child/children immunised. Parents were mainly concerned about possible side effects of the vaccine (66%), with only 0.2% being concerned about discussing a sexually transmitted disease with their children and 5% being concerned that use of the vaccine may lead to promiscuity.

Implications: Our findings suggest that public health education campaigns for HPV vaccination will find a majority of parents receptive to their children being vaccinated, but attention must be paid to appropriate explanation about HPV infection as the cause of cervical cancer and education about the safety of the HPV vaccine.

Key words: Human papillomavirus; vaccine; health knowledge, attitudes; immunisation; cancer, cervix; genital warts.

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Despite the success of cervical screening programs, there is still significant morbidity and mortality from cervical cancer in our community with approximately 800-1,000 new cases of cervical carcinoma diagnosed each year.[1,2] Cervical cancer is the 14th most common cause of cancer death in Australian women, with a lifetime risk of a woman developing cervical cancer of one in 130. Among Indigenous Australian women there is evidence of a higher rate of cervical cancer compared with non-Indigenous women, with a mortality rate nine times that of non-Indigenous women.[2] In addition, the burden of disease from cervical intraepithelial neoplasia (CIN) resulting from HPV infection is enormous, with 137,440 low-grade lesions and 104,395 high-grade lesions diagnosed between 1997 and 2004 in Australia.[3]

Human papillomavirus infection is the undisputed cause of cervical cancer. Approximately 20 high-risk oncogenic strains have been shown to be responsible for the majority of cases.[4-7] Although women are at risk of acquiring the virus and developing cervical cancer, both men and women may transmit the virus to their partner during sexual activity. Cervical infection with HPV is extremely common compared with the incidence of cervical cancer, with the majority of infections resolving over a six-month period.[8] Persistent infection is the precursor for development of pre-cancerous lesions. Strains HPV-16 and HPV-18 are the most prevalent high-risk, tumour-associated strains and are present in approximately 70% of cervical tumour specimens worldwide.[9] HPV is also the cause of anogenital tumours, laryngeal papillomatosis and genital warts,

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which may occur in both men and women.[10-12] The social and economic costs of HPV-induced diseases of the genital tract are huge and the development of prophylactic vaccines has been an important initiative.

Vaccines against the high-risk types HPV-16 and HPV-18 have been shown to be safe and immunogenic in previous trials and have been shown to prevent HPV-16/18 incident infection (91% efficacy for the quadrivalent vaccine (Merck) and 92% efficacy for the bivalent vaccine (GlaxoSmithKline) and 100% efficacy against persistent HPV-16/18 infection and CIN I, II and III up to four years post immunisation[13-15]). The quadrivalent vaccine has recently been licensed in Australia. Pre-teen and young adolescent women will be an important target population for immunisation, since it will be important to provide protection prior to onset of sexual activity and exposure to oncogenic HPV strains. HPV infection commonly occurs in young women around the time of first sexual encounter. Studies from the United States (US) have shown point prevalence ranges between 25% and 40% in young women, with a cumulative prevalence up to 82% in selected groups of adolescent women.[16] In a study of women attending university in the US who were initially HPV negative, 55% acquired HPV within three years.[17] Community acceptance of vaccination of young adolescent girls before they become sexually active will be paramount to achieve high coverage rates through successful immunisation programs.[16-18]

Awareness of the imminent availability of a HPV vaccine was raised with the nomination of Ian Frazer as Australian of the Year (2006) because of his involvement in the development of the vaccine. Concerns have more recently been raised in the media about the social implications of vaccinating adolescents to prevent a sexually transmitted disease and potentially cervical cancer. Because of improved coverage rates and resulting reduction in vaccine-preventable diseases, vaccine safety has become a predominant concern among immunisation providers and the community.[19,20]

Previous studies have shown that knowledge about the cause and prevention of cervical cancer is lacking and a successful education campaign will need to address this deficiency.[21] The implication that cervical cancer is linked to a sexually transmitted disease may lead to anxiety and concern about the use of HPV vaccine.[22]

Women's and adolescents' attitudes have been assessed in focus groups and as a component of HPV clinical trials as they are the most likely recipients of the vaccine.[22-30] However, future immunisation programs may include immunisation of men to improve herd immunity in the population.[31] Although licensing of the vaccine in the US did not include an indication for men, in Australia the vaccine is indicated for females 9-26 years of age and males 9-15 years of age. An assessment of men's attitudes to HPV vaccination in addition to the attitudes of women is essential to enable provision of appropriate education prior to a primary and/or catch-up immunisation program.

The aim of this study was to assess community attitudes in both men and women to the introduction of HPV vaccines in metropolitan and rural South Australia (SA). The methodology used was similar to that employed in a previous survey of community attitudes to the introduction of varicella vaccine.[32]

Methods

A cross-sectional study was conducted using a telephone survey of randomly selected households in SA. The survey was performed as part of the Health Monitor program through the Population Research and Outcomes Studies Unit, Department of Health, in SA.[33] The random sampling process used was based on the South Australian Electronic White Pages (EWP) telephone listings of households, both city and rural. An adult in the household, 18 years or older with the most recent birthday, was selected for an interview. The interviews were conducted using the computer-assisted telephone interviewing (CATI) methodology, which permits data obtained from the interviewer's screen to be entered directly into the computer database. A pilot study of 50 randomly selected households was conducted on 6 February 2006 to test question formats and sequence. Three thousand five hundred households were randomly selected from a total of 591,373 households in SA (Australian Bureau of Statistics (ABS), 2001 Census).[34]

Table 1: Household demographics (n=weighted data).

Household	Category	No. of resp.	Proportion of resp.
Age of respondent (10 year intervals) n=2,002	18-24 yrs	245	12.2%
	25-34 yrs	337	16.9%
	35-44 yrs	380	19.0%
	45-54 yrs	364	18.2%
	55-64 yrs	287	14.3%
	65-74 yrs	195	9.7%
	≥75 yrs	194	9.7%
Gender (n=2,002)	Male	981	49.0%
	Female	1,021	51.0%
Socio-economic status Postcode (SEIFA index of disadvantage measured in quartiles (n=1,975)	Lowest quarter	479	24.3%
	Second quarter	458	23.2%
	Third quarter	499	25.3%
	Highest quarter	539	27.3%
Highest educational qualification of interviewee (n=1,998)	Secondary school/studying	951	47.5%
	Trade	223	11.1%
	Certificate/ diploma	399	19.9%
	Bachelor degree	425	21.2%
Location of residential address (n=2,002)	Metropolitan	1,536	76.7%
	Rural	466	23.3%
Household income (n=1,686)	0-$20,000	292	14.6%
	$20,001-$40,000	412	20.6%
	$40,001-$60,000	324	16.2%
	$60,001-$80,000	260	13.0%
	>$80,000	398	19.9%
Country of birth (n=2,002)	Australia	1,576	78.7%
	Indigenous Aust.	13	0.6%
	UK	212	10.6%
	Other	201	10.0%

Note:
Proportions for each household characteristic may not add up to 100% due to rounding of figures to one decimal place.
Resp=respondents.

Participants were asked questions about the cause of cervical cancer followed by a comment that was read to them by the telephone interviewer to link the concept of a vaccine to prevent cervical cancer in women. "Cervical cancer is caused by Human Papilloma Virus which is a sexually transmitted virus that infects men and women. A vaccine called HPV vaccine will be available soon and should ideally be given to adolescents and young adults before they become sexually active." Further questions were asked to determine the level of acceptance and any concerns about introduction of a HPV vaccine program.

The survey data were weighted to the age, gender and geographical area profile (metropolitan or rural) of the population of SA and the probability of selection within a household. This methodology ensured that the survey findings were applicable to the SA population as a whole. Individual data were weighted by the inverse of the individual's probability of selection and then reweighted to benchmarks derived from the ABS estimated resident population (ERP) for 30 June 2004 (age, gender data) and 30 June 2003 (geographical area profile) for SA.[33,34] For questions regarding households rather than individuals, records were weighted by the inverse probability of the selection of the household then reweighted to benchmarks derived from the ABS 2001 Census of Population and Housing for occupied private dwellings by location.[33] Weighting was used to correct the distributions in the sample data to approximate those of the SA population. This is partly an expansion of the data and partly a matter of adjustment for both non-response and non-coverage, resulting in data that is representative of the population rather than limited to the households that responded. The Socio Economic Index For Areas (SEIFA) Index of Relative Socioeconomic Disadvantage was used as a measure of socio-economic status.[34]

Table 2: Causes of cervical cancer identified by household contacts, weighted to the population (single response).

Cause of cervical cancer suggested by interviewees (n=1,985)	Count	% (95% CI)
Don't know	1,562	78.7 (76.5-80.7)
Persistent HPV infection	42	2.1 (1.5-3.0)
Virus	140	7.1 (5.9-8.4)
Cell changes	61	3.1 (2.3-4.1)
Frequent sexual activity	32	1.6 (1.1-2.5)
Smoking	26	1.3 (0.8-2.3)
Sexually transmitted disease	24	1.2 (0.8-1.8)
Multiple partners	17	0.8 (0.5-1.4)
Sexual activity without protection	14	0.7 (0.4-1.3)
Sexually active at an early age	13	0.7 (0.4-1.2)
Poor hygiene	9	0.5 (0.2-0.9)
Stress	4	0.2 (0.1-0.7)
Other	41	2.1 (1.4-2.9)

Statistical analyses were performed with the Stata computer package using routines specifically designed to analyse clustered, weighted survey data.[35] Estimates of population percentages with 95% confidence intervals (95% CIs) are presented. Statistical tests were performed to assess significance at the confidence level of 0.05.

The study protocol was reviewed and approved by the Children Youth and Women's Health Service Human Research Ethics Committee, Adelaide, South Australia.

Results

Health Monitor survey

From 3,500 telephone numbers selected, 887 could not be contacted or were not household numbers. From the remaining 2,613 numbers, 2,002 interviews were conducted in February 2006, a participation rate of 76.6%.

Description of study sample (raw data)

Household demographic details were obtained. The median age of the household interviewee was 53.1 years (95% CI 52.3-53.8) compared with a median age of 38.5 years in the South Australia population (includes population <18 years of age). Of those interviewed, 852 were males (42.6% of the study population compared with 49% of the South Australian population, ABS 2004) and 1,150 were females (57.4% of the study population compared with 51% of the South Australian population, ABS 2004). Sixty-nine per cent (n=1,372) of households were situated in metropolitan Adelaide (compared with 73.3% of the SA population, ABS 2004) and 31.5% (n=630) were rural residences (compared with 26.7% of the SA population, ABS 2004). Fifteen interviewees refused to provide their age in years but agreed to identify an age category (see Table 1).

Description of weighted data

Weighting was performed on the raw data collected from the 2,002 randomly selected households in the Health Monitor Survey for both numbers and proportions. Including sampling weights in the analysis of the study population provides estimates that are unbiased in relation to the total population of SA. Within weighted households the mean age of the interviewee was 47.1 years (95% CI 46.1-48.1) with a near equal proportion of males (49.0%) and females (51%) (see Table 1). The study results are therefore based on a weighted survey sample of 981 males and 1,021 females. Six hundred and one household interviewees (30.1%) were parents/guardians of children in the household.

Community knowledge about the cause of cervical cancer

At the beginning of the interview an open-ended question was used where those interviewed were asked to identify the cause (viral) of cervical cancer. Almost 79% of interviewees were unable to nominate the cause. Two per cent (95% CI 1.5-3.0) correctly identified persistent HPV infection as the cause, a further 7.1 %

(95% CI 5.9-8.4) were aware of the viral aetiology and a further 10% were able to identify risk factors for the development of oncogenic disease (see Table 2).

As expected, women were more knowledgeable than men, with 61.4% (95% CI 52.9-69.3) of correct responses provided by women (χ^2_1=8.66, p=0.01). A difference in knowledge was also evident in relation to age with 15.2% (95% CI 11.6-19.8) of adults 45-54 years of age able to identify the cause as viral compared with only 2.9% (95% CI 0.9-9.5) of 18-24 year-olds and 5.3% (95% CI 2.8-9.5) of adults 75 years and older (χ^2_6=39.72, p=0.0003).

Educational attainment was an important factor in determining knowledge about the cause of cervical cancer with 20.9% (95% CI 16.6-26.0) who had attained a bachelor degree able to identify a viral cause compared with 10.7% (95% CI 7.9-14.3) who had attained a certificate or 6.0% (95% CI 3.2-11.3) who had attained a trade (χ^2_3=102.55, p<0.001). Households identified as of lowest economic status by use of the SEIFA scale of disadvantage were less informed (7.4% identified a viral cause (95% CI 5.2-10.3)) than those in the highest socio-economic group (13.0% identified a viral cause (95% CI 10.1-16.6); χ^2_3=12.81, p=0.02).

Community attitudes to use of HPV vaccine: who should receive it?

The majority (82.7% (95% CI 80.5-84.7)) interviewed stated that the HPV vaccine should be administered to both men and women to prevent cervical cancer in women (see Table 3). Equal proportions (p=0.70) of men (83.6% (95% CI 80.3-86.5)) and women (81.8% (95% CI 78.8-84.4)) agreed that an immunisation program should be targeted at both genders with only 6.9% (95% CI 5.6-8.5) stating that only women should receive the vaccine and 0.4% (95% CI 0.2-0.8) that only men should receive the vaccine. Almost 6% (95% CI 4.6-7.0) were undecided, 2.4% (95% CI 1.8-3.3) suggested the vaccine should not be given to anyone and the remaining 2.0% were classified as 'other'. This strongly positive result was equally supported across gender (p=0.70), age (p=0.57) and educational attainment (p=0.07).

Participants were asked at what age they felt it was appropriate to discuss and administer HPV vaccine. A mean age of 13 years and nine months (95% CI 13 years six months to 13 years 11 months) for males (n=1,751) and 13 years and nine months (95% CI 13 years six months to 13 years 11 months) for females (n=1,762) was identified as an appropriate age to discuss use of HPV vaccine, with a range of 5-50 years. Administration of the vaccine was considered appropriate approximately one year after this with a mean of 14 years and nine months (95% CI 14 years six months to 14 years and 11 months) for males (n=1,568) and 14 years and eight months (95% CI 14 years six months to 14 years and 11 months) for females (n=1,602), with a range of 3-40 years. Of those parents who provided an age, 95% agreed that the vaccine should be discussed and 92% agreed that it should be administered before 18 years of age for both males and females. Twelve per cent of the sample was unsure about when the vaccine should be discussed with adolescents and 21% was unsure about what age the vaccine should be administered. A higher proportion of those who were unsure about the appropriate age to discuss immunisation were over 65 years of age; 16.6% of ≥65 year-olds compared with 8.2% of 50-64 year-olds. Similarly for estimation of the most appropriate age to administer the vaccine, 27.8% of ≥65 year-olds compared with 21.4% of 50-65 year-olds were unsure, otherwise there was equal representation across other demographic variables.

Parental attitudes to use of HPV vaccine in children and adolescents

Of 2,002 households interviewed, 601 were households containing parents of children within the household. Seventy-seven per cent of parents interviewed agreed that their children should be immunised with HPV vaccine compared with 85.2% of parents who agreed that they should receive the vaccine for themselves for their own protection (χ^2_4=83.83, p<0.001). Sixty-nine per cent (95% CI 64.3-73.1) of parents agreed that this should include both sons and daughters with a further 6.6% (95% CI 4.6-9.4) suggesting only daughters and 1.4% (95% CI 0.7-2.8) suggesting only sons should receive the vaccine. A small proportion (5.4% (95% CI 3.6-8.0)) of parents considered that the decision should be made by the child/adolescent with a further 5.4 % (95% CI 3.6-8.1) claiming that their child/children should not receive the vaccine. Twelve per cent (95% CI 9.5-15.9) of parents remained unsure about whether their child should receive the vaccine.

There were no statistically significant differences observed in demographic details, apart from age, for parents who either agreed or disagreed to their child receiving the vaccine.

Respondents who agreed to receive the vaccine

Following provision of information on the cause and prevention of cervical cancer in women, almost 65% agreed they would

Table 3: Acceptance of HPV immunisation for males and females as reported by interviewees (weighted data).

Category n=1,975	Total number and proportion of adults			Number and proportion of females			Number and proportion of males		
	n	%	(95% CI)	n	%	(95% CI)	n	%	(95% CI)
Both males and females	1,634	82.7	(80.5-84.7)	827	81.8	(78.8-84.4)	806	83.6	(80.3-86.5)
Females only	136	6.9	(5.6-8.5)	75	7.5	(5.7-9.7)	60	6.3	(4.4-8.8)
Males only	8	0.4	(0.2-0.8)	7	0.7	(0.3-1.5)	1	0.1	(0.01-0.5)
No one	48	2.4	(1.8-3.3)	22	2.2	(1.5-3.2)	26	2.7	(1.7-4.3)
Other	39	2.0	(1.3-3.1)	16	1.6	(1.0-2.7)	22	2.3	(1.2-4.6)
Don't know	112	5.7	(4.6-7.0)	64	6.3	(4.6-8.5)	48	5.0	(3.7-6.8)

personally receive the vaccine (see Table 4). A higher proportion of women (73.4% (95% CI 70.2-76.3)) than men (67.9% (95% CI 63.9-71.6)) agreed they would personally receive the vaccine if it was available (χ^2_1=6.40, p=0.03). Younger respondents were also more likely to agree to vaccination with HPV vaccine than those who were older (92% for 18-24 year-olds compared with 73% for 45-54 years-olds). Using a logistic regression model a trend was identified; the higher the age of the interviewee the less likely they were to agree to be immunised with HPV vaccine (p<0.0005). In addition, interviewees who were married (p=0.001), male (p=0.027) and the least disadvantaged socio-economically (p=0.049) were most likely to decline immunisation with HPV vaccine. Of the total number of parents who agreed to receive the HPV vaccine, 93.1% (95% CI 91.2-94.6) also agreed that their children should be immunised. The majority (75.6% (95% CI 70.5-80.0)) of parents who would decline immunisation with HPV vaccine agreed, however, that their children should receive the vaccine.

Parental and community concerns about use of the vaccine

Parents and respondents overall identified that their main concern about use of the HPV vaccine was whether there were any side effects (see Table 5a and 5b). Other concerns included safety of the vaccine and the need for more education prior to a vaccine program being established. Respondents identified concern about receiving a vaccine that was not considered relevant to their current situation including being elderly, in a monogamous relationship, or not sexually active (see Table 5b). Concern about the use of the vaccine leading to promiscuity was indicated by 4.9% (95% CI 3.3-7.4) of parents (see Table 5a), with concern being more evident among mothers (6.2%) compared with fathers (3.3%). A slightly higher proportion of men (70.6%) were concerned about side effects of the vaccine than women (62.6%).

Similar causes of concern were identified by parents whether or not they agreed to immunisation for their children. There were significant differences in concerns identified between adults who agreed or did not agree to vaccination. Those who did not support immunisation with HPV cited reasons relevant to their low risk of contracting the infection rather than concern about side effects (16.1% of those who did not agree to vaccination compared with 49.3% of those who agreed to vaccination were concerned about side effects). Reasons given included not being sexually active (17.8% of those who did not agree to vaccination compared with 0.6% of those who agreed to vaccination), only having one partner (28% of those who did not agree to vaccination compared with 1.3% of those who agreed to vaccination) or too old (4.9% of those who would not agree to vaccination compared with 0.3% of those who agreed to vaccination).

Prevention of genital warts

The majority of participants (69.2% (95% CI 66.7-71.6)) agreed that they would be more likely to accept HPV vaccination if it also prevented genital warts (9.9% responded 'don't know' to this question). Although only a small proportion would refuse vaccination, 43.1% (95% CI 38.2-48.3) of those against vaccination with HPV agreed they would be more likely to accept vaccination if it also prevented genital warts. This was similar for both males (43.7% (95% CI 36.3-51.3)) and females (42.4% (95% CI 35.8- 49.2) p=0.23). There was no significant difference detected for demographic variables including degree of educational attainment or geographical location. However, there was a significant difference dependent on age of the interviewee (p<0.001). The elderly were less likely to be influenced in their decision by the addition of genital wart protection; 48.3% of interviewees over 75 years of age were more likely to accept HPV vaccination if it also protected against genital warts compared with 82.0% of 18-24 year-olds.

The Indigenous population

From a total of 2,002 households in metropolitan and rural SA, 13 people interviewed identified themselves as Indigenous. All respondents interviewed and identifying as being from Indigenous households agreed that HPV vaccine should be given to both men and women, with 10 of the 13 (77%) agreeing to receive the vaccine. Twelve of the 13 interviewed agreed they would be more likely to receive the vaccine if it also prevented genital warts. Only two households contained children and both respondents agreed to their children being immunised.

Table 4: Number and proportion of respondents who agreed to receive the vaccine and parents who agreed for their child/ren to receive the vaccine.

Household contact		Number and proportion of respondents who agreed to vaccination n=1,931			Number and proportion of parents who agreed for their children to be immunised n=601		
		n	% (95% CI)			n	% (95% CI)
Yes	Total	1,247	64.6 (62.0-67.1)	**Yes**	Both sons/daughters	414	68.9 (64.3-73.1)
	Females	657	52.7 (43.8-50.8)		Daughters	39	6.6 (4.6-9.4)
	Males	590	47.3 (43.8-50.8)		Sons	8	1.4 (0.7-2.8)
No		518	26.8 (24.6-29.2)			32	5.4 (3.6-8.1)
Don't know		166	8.6 (7.2-10.3)			74	12.3 (9.5-15.9)
Other						33[a]	5.4 (3.6-8.0)

Note:
(a) Decision to vaccinate should be the child's choice.

Discussion

Our results indicate that although there is a high acceptance of HPV immunisation in the community, only a small proportion of the community surveyed nominated HPV infection as the cause of cervical cancer. Studies conducted in the US have suggested a higher knowledge of HPV and cervical cancer than reported in our study.[22,25] The difference observed may be due to alternative study methodologies used to identify knowledge about HPV infection. Our results indicate that education about HPV infection and prevention needs to be directed towards the majority of the community but targeted towards those with least knowledge including men, young adults and the elderly, those with a trade or who have attained a certificate level of qualifications, and those who are the most disadvantaged in the community. Parents and adults require information about the disease and the vaccine in order to make an informed decision about whether they will consent to immunisation with the HPV vaccine. It is therefore essential for parents and adults to know and understand the association between HPV infection and the potential for developing cervical cancer. Studies have shown that providing a brief educational intervention about the association significantly improves parents' acceptance of the HPV vaccine.[22]

Acceptance of immunisation with HPV vaccine was only slightly higher in females than in males. Our results are similar to the acceptance rates observed in a study of parental attitudes to HPV vaccine by Brabin et al. conducted in the United Kingdom.[23]

The most socio-economically disadvantaged participants were more willing to accept HPV vaccination, which is a similar finding to a study examining acceptance of varicella immunisation prior to funding of the vaccine.[32]

Although concern was expressed about potential side effects of the vaccine particularly in children, adults who decided against vaccination identified they were in a low-risk group for acquiring the infection rather than having concerns about the vaccine itself. Similar concerns were expressed by men and women for the majority of responses, although some concerns expressed were gender specific such as concern about loss of libido (see Table 5b).

Our results confirmed that parents were not concerned about discussing sexually transmitted disease with their children and were willing to discuss use of the vaccine at an appropriate age. Parents who indicated they did not require the vaccine for themselves but would recommend it for their children were more likely to be married and in a monogamous relationship. This would suggest they did not consider themselves to be in an at-risk group but could see an advantage for their children. There was little evidence to suggest that anxiety about use of the vaccine leading to promiscuity was a concern. This compares favorably with results of a study conducted in Manchester, where 2.1% of parents surveyed suggested the vaccine should not be given because it would encourage promiscuity.[22] Estimates from studies in the US have determined that 24% of 15-year-old girls, 38% of 16-year-old girls and 62% of 18-year-old women have had sexual intercourse.[36] Providing the vaccine at 14 years of age (as an average estimate determined by adults in our study) would suggest a proportion of young women may not receive the vaccine until after exposure to HPV. Immunisation programs will need to be directed to younger adolescents to be more effective in preventing cervical cancer and adequate education will need to be provided to parents to ensure acceptance of vaccination at a younger age.

Understanding community concerns is essential to provide direction for education campaigns. Although concern may be expressed about side effects of the vaccine, reassurance can be provided that a local reaction is the only known significant side effect associated with use of HPV vaccine. This study provides baseline information for educators and policy makers as it represents the level of community understanding, concerns and acceptance of a HPV vaccine program.

Table 5: Concerns about receiving HPV vaccine.

a) Parental concerns about children receiving the HPV vaccine.

Main concern about child receiving HPV vaccine	Number and proportion of responses provided by interviewees n=599	
	n	% (95% CI)
Side effects of vaccine	397	66.4 (61.9-70.6)
Safety	30	5.0 (3.5-7.3)
Will lead to promiscuity	30	4.9 (3.3-7.4)
More education required	12	2.0 (1.0-3.9)
Having to discuss STDs	2	0.2 (0.02-1.1)
It can cause HPV infection	1	2.0 (1.0-3.9)
Anti-vaccination	4	0.7 (0.3-2.1)
Other	19	3.2 (1.8-5.6)
Don't know/not concerned	104	17.3 (14.2-21.1)

b) Respondents' concerns about receiving the HPV vaccine themselves.

Main concern about receiving HPV vaccine themselves	Number and proportion of responses provided by interviewees n=1,927	
	n	% (95% CI)
Side effects of vaccine	761	39.5 (36.8-42.2)
More information required	170	8.8 (7.4-10.5)
Not sexually active, don't need vaccine	102	5.3 (4.5-6.3)
Only have one partner, don't need vaccine	168	8.7 (7.3-10.3)
Safety	57	3.0 (2.2-4.0)
Sufficient/rigorous testing	46	2.4 (1.7-3.3)
Too old	30	1.5 (1.1-2.2)
Anti-vaccination	16	0.8 (0.4-1.9)
Other[a]	92	4.8 (3.7-6.2)
Don't know	438	22.7 (20.6-25.0)
It can cause cancer, it can cause HPV infection, cost		<1

Note:

(a) Included gender specific responses: Women: bird flu vaccine is more important, let nature takes its course, previous hysterectomy, would need more information. Men: afraid of needles, concern about loss of libido, not at risk always use condoms or have been circumcised or have good hygiene, provides a false sense of security.

The strength of this study is the large number of adults and parents randomly sampled from SA with a weighting process applied to the population to further improve the generalisability of the data. Previous studies have investigated parents' and women's attitudes to introduction of HPV vaccine whereas this study was a large-scale, community-based study that included men's knowledge, acceptance and concerns about the vaccine to provide protection against cervical cancer in women. This was a cross-sectional study and as such it has limitations in time, including the varying amounts of community education that have been provided about HPV infection during the past 12 months. At the time the study was conducted there was minimal information about HPV vaccine provided to the community and without a licensed vaccine promotional activity had not started. The telephone survey only allowed inclusion of English-speaking households because of the impracticality of providing interpreters. As non-English-speaking households represent a group that is at risk of poor access to educational materials, this group should be assessed using different methodology. Although a positive response to introduction of an HPV immunisation program was elicited, people may respond differently when faced with an actual vaccination decision.[37] Further information would need to be provided in order to obtain fully informed consent from individuals, such as the rapid clearance of most HPV infections within six months, a low rate of cervical cancer following HPV infection and alternative methods to avoid HPV infection.

Households randomised from listed telephone numbers may lead to bias as households without a land-line telephone or whose telephone numbers are not listed are excluded from the sample. In SA, it is estimated that 3% of households are not listed. The Indigenous population is over-represented in the unlisted group and therefore is under-represented in this study. Although households representative of the Indigenous population were few, acceptance of the vaccine was evident.

There are likely to be some difficulties in administration of a vaccine program for HPV. The initial target groups for immunisation are adolescents and young women, who are infrequent visitors to the general practitioner or primary care services. A school-based program is likely to be most effective in achieving high coverage. Another challenge for implementation of an HPV immunisation program may arise from a low perception of the need for the vaccine when the majority of incident HPV infections clear. Although now funded for 12-26 year-olds, the cost of the vaccine may be perceived to outweigh the benefit to the individual, particularly for those ineligible for funded vaccine. However, there appeared to be an enthusiastic response to the introduction of the vaccine and therefore appropriately targeted educational materials must be developed and made available to women and men of all ages.

Parents need to be reassured that although introduction of the vaccine will require discussion about its protective benefits against a sexually transmitted disease, this is unlikely to lead to a false sense of security and influence the future sexual behaviour of their children. Education for adults will be required to achieve adequate community levels of protection and will be essential to benefit from the effects of herd immunity in the community. Although cervical cancer is the most common form of HPV-related neoplasia, other anogenital cancers may eventually be eliminated by use of the vaccine in males as well as females. Educating men will be as important as informing women about the benefits of HPV vaccine if the ultimate goal is elimination of high-risk HPV infection from the community.

Conclusion

Community acceptance of HPV vaccine has been well established by the results of this study. However, linkages between health care and education systems to provide education about the benefits and availability of the HPV vaccine will be vital to achieve high levels of coverage. The future challenge for provision of this important vaccine will be to develop innovative funding strategies to ensure adequate vaccine delivery to populations with the highest mortality from this devastating disease, including our own Indigenous community.

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Pandemic Influenza and Community Preparedness

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Objectives. We aimed to examine community knowledge about and attitudes toward the threat of pandemic influenza and assess the community acceptability of strategies to reduce its effect.

Methods. We conducted computer-aided telephone interviews in 2007 with a cross-sectional sample of rural and metropolitan residents of South Australia.

Results. Of 1975 households interviewed, half (50.2%) had never heard of pandemic influenza or were unaware of its meaning. Only 10% of respondents were extremely concerned about the threat of pandemic influenza. Respondents identified children as the highest priority for vaccination, if supplies were limited; they ranked politicians and teachers as the lowest priority. Although only 61.7% of respondents agreed with a policy of home isolation, 98.2% agreed if it was part of a national strategy. Respondents considered television to be the best means of educating the community.

Conclusions. Community knowledge about pandemic influenza is poor despite widespread concern. Public education about pandemic influenza is essential if strategies to reduce the impact of the disease are to be effective. (*Am J Public Health.* 2009;99:S365–S371. doi:10.2105/AJPH.2008.153056)

The latest outbreaks of avian influenza A(H5N1) and novel H1N1 influenza have heightened concerns that an influenza pandemic is imminent.[1–4] In response, many governments have prepared protocols for rapid response and containment of infection to minimize the heavy burden of morbidity and mortality associated with previous pandemics.

Although epidemic influenza kills thousands of people worldwide each year, the emergence of influenza viruses with novel surface antigens poses a greater threat with increased economic and social consequences. The occasional crossover of the highly pathogenic but poorly transmissible avian influenza virus H5N1 into humans has placed governments on high alert for an influenza pandemic.

The United States began preparing pandemic influenza response plans in 1993 after the emergence of H5N1, closely followed by the European Union.[5,6] A worldwide response to emerging diseases coalesced in the World Health Organization (WHO) Global Outbreak Alert and Response Network, established in 2000.[7] Since then, a steady flow of WHO resource documents has encouraged the development of national pandemic influenza response plans and rapid action where outbreaks have occurred.[8]

Australia developed its own pandemic influenza management plan in 2005 in response to new cases of H5N1 in Asia; this was revised in 2006.[9] Each state has developed a plan that is consistent with the national plan but includes additional details relating to local circumstances.[10] The Australian federal government has invested more than AU $600 million in influenza pandemic preparedness,[11] including stockpiling antiviral drugs and personal protective equipment and developing a vaccine that is effective against H5N1.

In 2006 a national pandemic influenza exercise, Exercise Cumpston, carried out at sites across Australia, highlighted several gaps in existing plans, including poor communication with the general public and a lack of information targeted to indigenous and culturally diverse groups. The Exercise Cumpston report led to an emphasis on improved communication, including government engagement in increasing public knowledge, with the aim of building a base level of awareness and understanding across the community and among primary care providers about the threat of an influenza pandemic.[11]

Although many government and public health agencies have been involved in pandemic influenza planning, the wider population (including community and hospital health care workers) has generally not been included in decision-making on issues that will require community compliance. Public health control measures that are inadequately understood or supported by communities may fail to be implemented.[12] Poorly understood control measures caused confusion and fear during the SARS (severe acute respiratory syndrome) outbreak.[13] Pandemic influenza planning in North America has included a US–Canada summit,[14] the Public Engagement Pilot Project on Pandemic Influenza,[15] and local initiatives such as the Baltimore program B'More Prepared,[16] but citizen consultation and engagement have been limited. In New Zealand, consideration of ethical issues in pandemic influenza planning included elements of community consultation.[17]

Engagement of the community as active participants in pandemic flu preparedness is considered essential if a successful prevention program is to be established.[17,18] We aimed to assess community knowledge regarding pandemic influenza preparedness and acceptance of government strategies to reduce the impact of pandemic influenza in South Australia.

METHODS

We conducted a cross-sectional telephone survey of randomly selected households in South Australia (population 1.5 million). The study was part of the Health Monitor program of the Population Research and Outcomes Studies Unit, Department of Health, South Australia.[19] We based the random sampling process on the South Australian electronic white pages household telephone listings, both city and rural. The household contact identified the adult in the household (aged ≥18 years) who most recently had a birthday; up to 10 callbacks were made to interview the identified individual. The interviews were conducted by the computer-assisted telephone interviewing method.

In South Australia, 97% of households have a telephone listed in the white pages. Phone calls were made at different times of the day and evening and on different days of the week.

A pilot study of 50 randomly selected households was conducted in March 2007 to test question formats and sequence prior to commencement of the main study, which took place in April and May 2007. The method was similar to that employed in previous community surveys.[20,21] Questions were intended to determine the level of knowledge and community acceptance of government strategies for pandemic influenza control. The complete list of survey questions is available from the corresponding author on request.

The survey data were weighted to the age, gender, and geographical area profile of the population of South Australia and the probability of selection within a household. Individual data were weighted by the inverse of the individual's probability of selection and then reweighted to benchmarks derived from the Australian Bureau of Statistics' estimated resident population for June 30, 2005 (age, gender data, and geographical area profile), for South Australia.[19] For questions regarding households, records were weighted by the inverse probability of the selection of the household, then reweighted to benchmarks derived from the Australian Bureau of Statistics' 2001 Census of Population and Housing. We used the Socioeconomic Index for Areas Index of Relative Socioeconomic Disadvantage as a measure of socioeconomic status.[22]

We used Stata software for statistical analyses, with routines specifically designed to analyze clustered, weighted survey data.[23] Statistical tests were 2-tailed, with a significance level of 5%.

RESULTS

Of 3900 telephone numbers selected, 960 could not be contacted or were not household numbers. From the remaining 2940 numbers, 1975 interviews were conducted, a participation rate of 67.2%.

The mean age of the household interviewees was 53.4 years, with a median of 53 years (95% confidence interval [CI]=52, 54) and a range of 18 to 94 years. We weighted the raw data collected from the 1975 randomly selected households in the Health Monitor Survey for both numbers and proportions (Table 1). Within weighted households the mean age of the interviewee was 47.2 years (95% CI=46.2, 48.2), with a nearly equal proportion of men (49.1%) and women (50.9%; Table 1). Our results were therefore based on a weighted survey sample of 969 men and 1006 women. Children younger than 18 years resided in 686 (34.7%) of the households interviewed.

Knowledge of Pandemic Influenza and Prevention

Of 1975 households interviewed, 50.2% of the respondents had either never heard of pandemic influenza (8.4%; 95% CI=7.0, 10.1) or were unaware of its meaning (41.8%; 39.1, 44.5). When asked an open-ended question about the meaning of pandemic influenza, 34% were able to provide the meaning.

A correct response (WHO definition[24]) included recognition of a global spread of influenza with potential to cause a large number of deaths. A statement based on the WHO definition of pandemic influenza was read to all respondents after they answered the question on knowledge of pandemic influenza to facilitate further discussion. Age was a statistically significant correlate of knowledge of pandemic influenza ($\chi^2_6=49.543$; $P<.001$): respondents aged 45 to 64 years were likeliest to know of pandemic influenza, and those aged 18 to 24 years or older than 75 years were least likely to possess such knowledge.

Participants with a higher level of educational attainment were more likely to know the meaning of pandemic influenza ($\chi^2_3=91.817$; $P<.001$). Those with secondary school education were less likely to know the meaning of pandemic influenza (29.7%; 95% CI=26.6, 33.1) than were respondents who had learned a trade or served an apprenticeship (35.0%; 95% CI=27.7, 43.1) or who had completed a bachelor's or higher degree (56.7%; 95% CI=50.3, 62.9).

The test for trend showed that the proportion of respondents who provided the correct meaning of pandemic influenza increased by 4.3% (95% CI=2.0, 6.5) with each quartile of socioeconomic status ($P<.001$). Almost one third of respondents (31.5%; 95% CI=27.1, 36.2) in the lowest socioeconomic quartile knew the meaning of pandemic influenza; 46.5% (95% CI=41.7, 51.5) of those in the highest socioeconomic quartile answered this question correctly.

The mean level of concern regarding pandemic influenza (on a scale of 1–10) was 5.8 (95% CI=5.7, 6.0; Figure 1). Thirty percent of respondents had a score of 7 or higher, suggesting a high level of concern in the community, with 10% of respondents indicating extreme concern about the threat of pandemic influenza with a score of 10. A test for trend showed that women were more likely than men to report high concern about the threat of pandemic influenza ($P<.001$). The mean score for concern among men was 5.5 (95% CI=5.3, 5.7); for women it was 6.1 (95% CI=6.0, 6.3). The gender difference in means was 0.65 (95% CI=0.36, 0.93).

As age increased and household income decreased, the proportion of respondents concerned about pandemic influenza increased. A test for trend ($P<.001$) showed that as age increased (by 10-year categories), the level-of-concern score increased by 0.39 (95% CI=0.31, 0.47). Similarly, as household income decreased (household income categories shown in Table 1), the score for concern increased by 0.37 (95% CI=0.45, 0.29). Among lower-income households (<AU $20 000), 78.9% (95% CI=73.9, 83.1) were concerned about pandemic influenza (scores of 5–10); among respondents with higher household incomes (>AU $80 000), 56.0% (95% CI=50.0, 61.9) were similarly concerned.

The majority of respondents believed that the routine influenza vaccination would give sufficient protection against a pandemic influenza strain. This was considered true by 52.4% (95% CI=49.7, 55.1) of respondents; 27.5% (95% CI=25.2, 29.9) were aware that the influenza vaccine would not provide protection, and 20.0% (95% CI=17.9, 22.3) were unsure. Respondents with higher educational attainment were less likely to believe that the annual influenza vaccine would protect against a pandemic strain (45.3%; 95% CI=38.9, 51.8); 56.6% of those with lower educational attainment held this opinion (95% CI=53.0, 60.2). A test for trend was significant ($P<.001$): 10.1% fewer respondents (95% CI=6.4, 13.8) believed in the efficacy of the annual influenza vaccine for each increase in educational attainment (categories described in Table 1).

TABLE 1—Household Demographics of Survey Respondents (n = 1975): South Australia, 2007

Respondent Characteristics	No. of Respondents, Raw (Weighted)	Respondents, Weighted %
Age, y		
18–24	107 (241)	12.2
25–34	174 (326)	16.5
35–44	336 (369)	18.7
45–54	411 (360)	18.2
55–64	383 (291)	14.7
65–74	289 (193)	9.8
≥75	275 (195)	9.9
Gender		
Men	789 (969)	49.1
Women	1177 (1006)	50.9
Socioeconomic status,[a] quartile		
First	581 (563)	28.7
Second	464 (441)	22.5
Third	387 (392)	19.9
Fourth	529 (568)	28.9
Educational attainment[b]		
Secondary school/studying	1076 (1022)	51.9
Trade/certificate/diploma	549 (581)	29.4
Bachelor's degree	345 (369)	18.7
Residence		
Metropolitan	1319 (1476)	74.7
Rural	656 (499)	25.3
Household income, AU $		
≤20 000	448 (324)	16.4
20 001–40 000	366 (346)	17.5
40 001–60 000	286 (306)	15.5
60 001–80 000	254 (263)	13.3
>80 000	385 (440)	22.3
Declined to answer	236 (297)	15.0
Country of birth		
Australia	1536 (1574)	79.7
United Kingdom	244 (205)	10.4
Other	195 (197)	10.0
Employment		
Full time	721 (853)	43.2
Part time	379 (371)	18.8
Home duties	132 (128)	6.5
Retired	612 (437)	22.1
Unemployed	131 (186)	9.4

Note. Data were weighted to the South Australian population.
[a]The measure was the Socioeconomic Index for Areas Index of Relative Socioeconomic Disadvantage, in quartiles from lowest to highest.
[b]For this category, n = 1972.

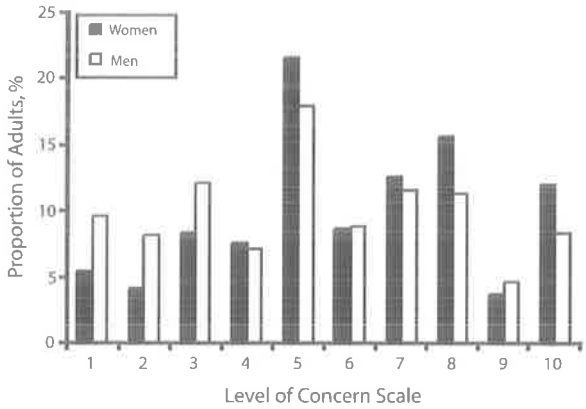
Preparation for Pandemic Influenza

Use of antiviral drugs. If antiviral drugs were available, 49.3% (95% CI=46.6, 52.0) of respondents said that they would not buy and store them in preparation for an influenza pandemic, 35.0% (95% CI=32.5, 37.6) stated that they would store antiviral drugs, and 15.7% (95% CI=13.8, 17.7) were unsure. Women, households with lower income, respondents with a lower level of educational attainment and of lower socioeconomic status, and residents of rural areas were all significantly more likely to buy and store antiviral medication in preparation for an influenza pandemic (Table 2).

Acceptance of a pandemic influenza vaccine. Of all households interviewed, 81.4% (95% CI=79.2, 83.5) said that once a licensed vaccine to prevent pandemic influenza was available, they would agree to be vaccinated; 11.7% (95% CI=10.0, 13.5) reported that they would not agree to be vaccinated; and 6.9% (95% CI=5.6, 8.6) were unsure. Similarly, when parents (n=578) were asked if they would have their children vaccinated with a licensed vaccine to prevent pandemic influenza, the majority agreed that their children should receive the vaccine (78.6%; 95% CI=74.3, 82.4). Some parents (8.1%; 95% CI=5.7, 11.3) would refuse vaccination for their children, and others (8.1%; 95% CI=6.1, 10.7) were unsure.

The highest-priority recipients of vaccine suggested by respondents if supplies were limited were children (49.7%; Table 3). We observed a gender difference in this response ($\chi^2_1=25.062$; $P<.001$): men were more supportive of emergency service workers receiving priority for vaccination (63.0%; 95% CI=56.1, 69.4) than were women (37.0%; 95% CI=30.6, 43.8). Women were more likely to consider vulnerable groups such as children, the elderly, or sick people as needing a priority for vaccination (53.9%; 95% CI=50.9, 56.9) than were men (46.1%; 95% CI=43.1, 49.1).

Wearing a mask to prevent spread of infection. A high proportion of respondents (83.5%; 95% CI=81.3, 85.5) would agree to wear a mask to prevent spread of infection in pandemic influenza. Women, older adults (aged >50 years), part-time employees, and adults with home duties were most willing to wear a



Note. Data were weighted to South Australia population. Participants were asked, "Could you rate your current level of concern about pandemic flu on a scale of 1-10 where 10 = extremely concerned and 1 = not at all concerned?"

FIGURE 1—Level of concern about pandemic influenza among men and women.

mask (Table 4). The responses provided by indigenous participants were similar, with 19 of 20 agreeing to wear a mask.

Reduction of virus transmission. Of adult employees interviewed, 61.7% (95% CI=58.4, 64.9) said they would stay home from work if they had symptoms suggestive of influenza. A small proportion of respondents (3.6%; 95% CI=2.7, 4.8; n=52) worked from home and were not included in any further analysis of home isolation. The proportion of respondents who would miss work if they were ill rose substantially (98.2%; 95% CI=97.1, 98.9) if this was recommended as part of a national strategy to prevent the spread of infection. Men were less likely than women to stay at home if unwell (χ^2_1=36.842; P<.001): 56.0% (95% CI=51.0, 60.9) and 72.6% (95% CI=68.4, 76.4), respectively.

Full-time employees were less likely to stay at home with influenza-like symptoms (56.9%; 95% CI=52.2, 61.4) than were part-time employees (74.4%; 95% CI=68.5, 79.5; χ^2_1=27.337; P<.001). When home isolation was presented as a component of a national strategy, we observed no significant gender difference. Employees with a higher income (>AU $80 000) were less likely to stay at home with an influenza-like illness (55.0%; 95% CI=48.5, 61.3) than were those with an income under AU $20 000 (74.3%; 95% CI=64.0, 82.5). A test for trend was significant (P<.001): as income increased by multiples of AU $20 000, the proportion of respondents who said they would stay home decreased by 4.9% (95% CI=2.5, 7.3).

Community Attitudes Toward Preparedness

Fewer than one third of respondents (32.0%; 95% CI=29.5, 34.6) believed that enough was being done to prepare for pandemic influenza; 44.7% (95% CI=42.1, 47.4) were unsure.

Respondents with higher educational attainment were more concerned that not enough was being done, with 66.5% (95% CI=58.4, 73.8) suggesting that more should be done; 59.3% (95% CI=54.3, 64.2) of participants with secondary schooling shared this concern. More than 40% of respondents agreed that further information and education needs to be provided to the public. Other priorities suggested were vaccination (18.3%; 95% CI=16.4, 20.4), including compulsory vaccination, and increased funding for vaccine research (7.1%; 95% CI=5.3, 9.5).

More than one third of respondents (36.6%; 95% CI=34.1, 39.3) received most of their information from television, and 16.9% (95% CI=15.1, 18.8) from newspapers. Doctors' waiting rooms provided most information for 4.4% of respondents (95% CI=3.5, 5.6); only 1.2% (95% CI=0.8, 1.9) cited the Internet as the most important source of information.

Most respondents (69.6%; 95% CI=67.1, 72.0) considered television the best means of communication about pandemic influenza; 12.4% (95% CI=10.7, 14.2) cited radio. An information pack from the government was considered a priority by 2.8% of participants (95% CI=2.1, 3.8), and another 1.6% (95% CI=1.1, 2.4) suggested pamphlets should be sent by mail (n=1975). Almost all (97.4%; 95% CI=87.3, 99.5) younger adults (<55 years of age) cited the Internet as the best means of communication. In addition, 67.9% (95% CI=65.1, 70.5) of respondents who identified television as the best means of communication were younger than 55 years. Older adults (≥55 years) were more likely to cite their doctor's office: 81.7% of respondents who relied on this information source were in this age group. Men were more likely to suggest the Internet as a source of information (75.0%; 95% CI=43.3, 92.2), and women were more likely to cite the doctor's office as the best source (82.7%; 95% CI=57.0, 94.5). Women preferred information packs (62.6%; 95% CI=47.0, 75.9) and letters (75.5%; 95% CI=53.5, 89.1).

DISCUSSION

Despite initial widespread publicity regarding pandemic influenza and advocacy to build a base level of awareness and understanding among the population,[26] we found that the majority of adults in the community we surveyed were completely unaware of the possibility of pandemic influenza and harbored misconceptions about protection against a pandemic influenza strain. This was especially true for young adults and the elderly, which is noteworthy because the elderly are at increased risk of complications and mortality from influenza. We observed a high level of concern within the community, particularly among elderly women and adults in low-income households, possibly reflecting perceived vulnerability to potential loss of income and concern for dependents. Community knowledge about pandemic influenza is deficient, and exploration of the most effective methods for providing information to the general public is urgently needed.

Knowledge of Pandemic Influenza Preparedness

Socioeconomically disadvantaged and low-income respondents expressed a higher level of concern, which may reflect perceived lack of

TABLE 2—Respondent and Household Characteristics Significantly Associated With Willingness to Buy and Store Antiviral Drugs in Preparation for a Pandemic Influenza: South Australia, 2007

Characteristics	No. of Respondents, Weighted	% (95% CI)	χ^2 (*P*)
Gender (n = 1664)			6.411 (.039)
Men	317	45.9 (41.3, 50.5)	
Women	374	54.1 (49.5, 58.7)	
Socioeconomic status,[a] quartile (n = 1657)			22.368 (.001)
First	233	49.8 (44.2, 55.5)	
Second	153	41.7 (36.0, 47.7)	
Third	117	34.9 (29.0, 41.3)	
Fourth	183	37.5 (32.5, 42.8)	
Educational attainment (n = 1661)			13.231 (.014)
Secondary school/studying	382	44.4 (40.5, 48.3)	
Trade/certificate/diploma	208	42.2 (36.7, 47.9)	
Bachelor's degree	100	32.5 (26.4, 39.2)	
Residence (n = 1664)			6.202 (.037)
Metropolitan	489	39.7 (36.4, 43.2)	
Rural	202	46.6 (41.1, 52.2)	
Household income, AU $ (n = 1422)			19.417 (.009)
≤ 20 000	116	44.6 (38.5, 50.8)	
20 001–40 000	138	48.7 (41.7, 55.7)	
40 001–60 000	110	42.0 (34.9, 49.5)	
60 001–80 000	90	37.7 (30.9, 45.0)	
> 80 000	126	33.3 (27.6, 39.4)	

Note. CI = confidence interval. Data were weighted to the South Australian population. Respondents were asked, "When antiviral medications are available will you buy and store these in preparation for a flu pandemic?"
[a]The measure was the Socioeconomic Index for Areas Index of Relative Socioeconomic Disadvantage, in quartiles from lowest to highest.

resources in times of stress. This concern may relate to the potential for loss of income or job if they comply with government recommendations, such as home isolation or quarantine, as shown by Blendon et al.[27] Our findings and those of other studies suggest that educational interventions are required to improve trust in the community and to promote effective coping mechanisms that could support implementation of government strategies.[28,29]

As a component of a pandemic influenza education campaign, the community must be made aware that the routine influenza vaccine will not provide protection against a novel strain and that an effective vaccine will need to be developed.[30,31] In addition, the community will need to be aware that because of delays in the provision of a vaccine, other preventive measures will be required to minimize the spread of infection, many of which could be promoted for use during seasonal outbreaks.

Acceptance of Government Preparedness Strategies

Support for development of a vaccine to prevent pandemic influenza is strong.[18] H5N1 vaccines are licensed in several countries, and policymakers are considering vaccinating their communities with a prepandemic influenza vaccine in the near future, followed by a booster pandemic vaccine when it becomes available. A vaccine uptake of 80% in the community is likely necessary to provide herd immunity during an outbreak. Our results indicate that most citizens would agree to be vaccinated, but almost 12% would refuse vaccination, meaning that 2.4 million adults would remain unprotected and would be able to spread infection.

Compulsory vaccination was supported by a small proportion of our respondents. It has been debated and rejected in Australia for routine immunizations but has been accepted in some states in the United States.[32] The community may consider that some circumstances warrant compulsory immunization, such as a global threat of disease with high morbidity and mortality. The most vulnerable groups within society, selected by our respondents as a priority for vaccination, included children, the elderly, and the ill. Children are also an important priority for vaccination in government plans, to control the spread of infection, particularly to the elderly.[33–35]

Rationing of antivirals and vaccines is controversial.[36] The Australian government places the highest priority on the protection of providers

TABLE 3—Respondents' Ranking of Groups for Priority for Vaccination to Protect Against Pandemic Influenza if Vaccine Supplies Were Limited: South Australia, 2007

Priority for Vaccination	Respondents, No.	% (95% CI)
Children	981	49.7 (47.0, 52.3)
Elderly	461	23.3 (21.1, 25.7)
Hospital workers	172	8.7 (7.3, 10.3)
Sick people	104	5.3 (4.2, 6.6)
Doctors	40	2.0 (1.4, 3.0)
Emergency workers	39	2.0 (1.4, 2.3)
Parents	19	1.0 (0.6, 1.7)
Pregnant women	5	0.3 (0.0, 0.6)
Police	4	0.2 (0.1, 0.6)
Politicians	3	0.2 (0.1, 0.6)
Teachers	2	0.1 (0.0, 0.4)
Other[a]	79	4.0 (3.1, 5.2)
Don't know	57	2.9 (2.0, 4.2)
Declined to answer	9	0.5 (0.2, 1.0)

Note. CI = confidence interval. Data were weighted to the South Australian population. Sample size was n = 1975. Respondents were asked, "If supplies of vaccine are limited who in the community do you think should receive the vaccine first?"
[a]Includes single individual responses or suggestions of specific age groups (aged 15–20 years, ≤ 21 years), or other groups (Aboriginal people, city people, mothers and grandmothers, people of low socioeconomic background, farmers, persons randomly picked from the population, those who can least afford it), or "it doesn't matter."

TABLE 4—Willingness to Use a Mask to Prevent Spread of Influenza During an Influenza Pandemic by Age, Gender, and Employment Status: South Australia, 2007

Respondent Characteristics	Respondents, Weighted, No.	% (95% CI)	χ^2 (*P*)
Gender			8.333 (.018)
Men	786	86.9 (83.8, 89.4)	
Women	862	91.0 (88.7, 93.0)	
Age, y			10.043 (.014)
18–49	897	87.0 (84.0, 89.5)	
50–64	416	92.2 (89.5, 94.3)	
≥65	335	90.6 (87.01, 93.3)	
Employment			14.089 (.043)
Unemployed	137	87.7 (78.7, 93.2)	
Retired	384	91.0 (88.0, 93.4)	
Home duties	113	96.0 (89.6, 98.5)	
Part time	314	90.5 (86.5, 93.5)	
Full time	701	86.5 (83.1, 89.3)	

Note. CI = confidence interval. Data were weighted to the South Australian population. For all categories, sample was n = 1852. Respondents were asked, "Would you agree to wear a mask if advised that this was a way of preventing spread of the infection?"

of health and emergency services.[18,37] The difference in government and community priorities will need to be addressed to encourage community participation in strategies to prevent pandemic influenza. The mechanism by which rationing of vaccines is communicated and implemented will be vital in determining public trust and reaction.

A majority of our respondents indicated a willingness to stay home from work with symptoms of influenza-like illness if required as part of a national strategy to stop the spread of infection. During an influenza pandemic, home isolation may be required for up to a week,[37] and our data suggest that this strategy would be supported by the community. Wearing masks is considered a high priority by the Australian government, which has designated significant funding in its pandemic influenza preparedness plans for fitting masks to all health care workers. Our study showed robust community acceptance of wearing masks, although no further details, such as how long masks would need to be worn or their efficacy in preventing transmission, were discussed during our interviews.

Our respondents expressed a need for more pandemic influenza preparedness. This included providing more information, and they designated television as the most appropriate medium for communication. Most available information is provided on the Internet by government agencies, but only a small proportion of our participants had accessed this information; it may therefore not be the optimal way to inform the community. Our respondents also identified funding for pandemic influenza research and development of a vaccine as high priorities. Informing and engaging the community could lead to community lobbying of government or to community funding for vital pandemic influenza research.

Limitations

Although our results may be used to inform pandemic policy, the community may respond differently when the threat of a pandemic is imminent. The community's response is also likely to be affected by the perceived effectiveness of government strategies during a pandemic and by a clear and consistent release of information to the public, neither of which was measured during our study.

Nonresponse in telephone surveys may result in underrepresentation of subgroups within the population. This may be important because of the high mortality in younger age groups in previous pandemics.[25] Weighting the survey data may compensate for this bias because it helps ensure that the age and gender structure of the sample better represents that of the general population. This type of survey does not provide an opportunity to give participants a detailed explanation of the measures presented, including risks and benefits, which might evoke a different response.

Conclusions

Accurate information must be provided to the public both before and during a pandemic on how to care for those infected and how to protect against the spread of infection. Provision of information in a clear, accessible, and engaging way is required to optimize community acceptance of public health actions to prevent or respond to a pandemic.[38] Strategies to restrict the spread of disease will be ineffective if communication is not improved. Although the Exercise Cumption report acknowledged that communication to the public was inadequate, little has been done to raise community awareness.

Community and government need to work as partners in planning for a pandemic. The needs of vulnerable groups within the community who may be severely affected should be considered. Clarity and transparency of decision-making, along with thorough and efficient communication of information to the public, are essential to the mission of pandemic influenza preparedness—prevention of a global catastrophe. ■

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Contributors

H. Marshall and P. Ryan designed and conducted the study, analyzed the results, and are joint authors of this article. H. Marshall is the primary author and assumed the primary role for preparation of the article. D. Roberton, J. Street, and M. Watson helped interpret the results. All authors contributed substantially to writing

and reviewing drafts of the article and approved the final version.

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Human Participation Protection

The study was approved by the Children, Youth and Women's Health Service research ethics committee.

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Viewpoint

Should routine childhood immunizations be compulsory?

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Abstract: Routine childhood immunizations are compulsory in a small number of countries, including the United States of America. Arguments used to justify making immunizations compulsory include enhancing the health of the community and treating as paramount the rights of the child to be protected against vaccine-preventable diseases. But compulsory immunization infringes the autonomy of parents to make choices about child rearing, an autonomy which we generally respect unless doing so seriously endangers the child's health. We present a historical review and ethics discussion on whether routine childhood immunizations should be compulsory. We conclude that, for both ethical and practical reasons, routine immunization should not be compulsory if adequate levels of immunization can be achieved by other means.

Key words: bio-ethics; coercion; ethics; no fault compensation.

Childhood immunization is one of the most important and cost-effective public health measures in our armoury. There is no doubt that immunization reduces the incidence and severity of infectious diseases and saves lives.[1–3] But vaccines are unique among public health measures, in that their administration occasionally causes injury. Should routine childhood immunizations be compulsory? In this paper we consider primarily those immunizations currently recommended in the Australian routine childhood schedule.[2]

HISTORICAL PERSPECTIVES

Compulsory immunization: past and present

There is nothing new about compulsory immunization, nor about vociferous anti-vaccination movements. The British Vaccination Act of 1853 made smallpox vaccination compulsory for all infants in the first 3 months of life, and made defaulting parents liable to a fine or imprisonment.[4] This was the first time that public laws potentially infringed civil liberties, and the Act spawned an Anti-Compulsory Vaccination League and an anti-vaccination demonstration in Leicester attended by 100 000 protesters.[4] Vaccination rates fell and, in 1898, 'conscientious objectors' were excused from having their children vaccinated.

The United States of America has an even longer history of compulsory immunization. The State of Massachusetts introduced compulsory smallpox vaccination in 1809, while in 1922 the Supreme Court upheld laws requiring vaccination for school entry.[5]

Routine childhood immunization, against at least some infectious diseases, is compulsory in a number of countries, including Croatia, France, Italy, Poland, Slovakia and Taiwan. The United States of America has had school immunization laws, requiring compulsory immunization at entry to licensed day care and to school, for a number of years, although enforcement has been variable. In the 1970s unimmunized children were excluded from school during measles outbreaks. In 1977, a measles outbreak occurred in Los Angeles County. After immunizing thousands of students, 50 000 of the 1.4 million students remained unimmunized and were excluded from school. Most returned within days with proof of immunity, and the number of measles cases plummeted.[5] Recently there has been greater emphasis on enforcing immunization requirements at school entry. There is no national immunization law, all regulations being State-based. The exact requirements vary but all States require diphtheria, measles, polio and rubella immunization. Sanctions for non-compliance also vary, and some States threaten to take child care proceedings if there is persistent failure to immunize. Forty-eight of the 50 States allow exemptions for those with deeply held religious beliefs opposed to immunization, but only 15 States allow parents to decline immunization for 'philosophic' reasons.[5] It is argued that US school immunization laws mainly act as a safety net to ensure that under-privileged children are immunized, while offending 'very few', although up to 2.5% of students are exempted in States which allow philosophic exemption.[5]

FACTUAL CONSIDERATIONS

Vaccines save lives; failure to immunize costs lives

The eradication of smallpox in the 1970s, by targeted use of smallpox vaccine, has not only prevented many thousands of deaths, but is estimated to have saved US$1.2 billion annually in the 25 years since the last case was reported.[3] Poliomyelitis has almost been eradicated from the world.[6]

More recently conjugate vaccines have reduced the annual number of cases of Haemophilus influenzae type b (Hib) infection, previously the commonest cause of meningitis in industrialized countries, by over 95%.[7] Most routine childhood

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vaccines protect against communicable diseases, which can be transmitted person-to-person, and which we will term **transmissible**. Immunization against transmissible infections, for example, diphtheria, pertussis, polio, Hib, hepatitis B, meningococcus, pneumococcus, varicella, protects the child, but also reduces spread to other children and adults, resulting in **herd immunity** (see below). Universal rubella immunization is a special case, where the direct benefit is primarily to persons other than the recipient, by reducing the incidence of congenital rubella syndrome. In contrast, tetanus immunization protects only the recipient, because tetanus is not transmissible.

It is sometimes argued that vaccine-preventable diseases are no longer as serious, and that modern medical treatment would prevent the high morbidity and mortality once seen. A recent example illustrates the fallacy of this belief:

The break-up of the Soviet Union caused enormous disruption to health services. As a result rates of childhood immunization fell drastically. Between 1991 and 1996 there was an outbreak of diphtheria, with over 140 000 cases notified and over 4000 deaths.[8]

Vaccine-preventable diseases remain life-threatening, and outbreaks will recur if immunization levels fall.

Immunizations can be harmful

Although the commonest adverse events following immunization are relatively minor and self-limiting, such as local reactions, fever and irritability, immunizations can occasionally cause severe irreversible complications and rarely, even death.

Vaccine-associated paralytic poliomyelitis (VAPP) is estimated to occur once in every 2.4 million doses of oral poliovirus vaccine (OPV).[9] Measles vaccine causes an acute encephalitis with an incidence of one in a million doses, although in contrast the incidence of acute encephalitis after wild-type measles infection is about one in a thousand.[10] Yellow fever vaccine has caused yellow fever in a small number of recipients, and six deaths have been reported from fulminant yellow fever acquired from the vaccine.[11]

ETHICAL CONSIDERATIONS

Risks versus benefit

We take calculated risks every day of our lives. Travel is a good example. The speed and convenience of road, rail and air travel mean that most persons accept the slight risk of an accident in favour of the benefits offered by quicker travel.

In general the benefits of immunization far outweigh the risks. The risk of vaccine-induced injury is hundreds to thousands of times lower than the risk of similar complications of the natural, wild-type infection.[1–3]

People who are afraid of harming their children by immunization tend to over-emphasize the risks of vaccine injury and to minimize the risk of wild-type disease.[2] This reflects a general tendency to be more worried about causing damage to one's child by doing something to them than by not doing it. This is referred to as the fear of commission rather than of omission.[2] In his autobiography, Benjamin Franklin wrote with tragic eloquence:

> In 1736, I lost one of my sons (Francis Folger) a fine boy of 4 years old by the smallpox. I long regretted bitterly and still regret that I had not given it to him by inoculation. This I mention for the sake of parents, who omit the operation on the supposition that they should never forgive themselves if the child died under it: my example shows the regret may be the same either way, and that therefore, the safer should be chosen.

Public health and paternalism

Some public health interventions that have been shown to prevent injury or death have been made compulsory, because the public cannot be trusted to comply unless there is a degree of coercion. Examples include seat-belt legislation, motor-cycle helmets, bicycle helmets and swimming pool fences. Sanctions for disobeying regulations are usually fines, and possible loss of motor vehicle licence for frequent offenders regarding seat belts and motor-cycle helmets. Those who oppose such legislation do so for different reasons, such as because it is paternalistic, but they also try to argue that the intervention may itself be harmful. Seat belts can occasionally cause crush injuries to the chest or spine, and while being thrown from a car is likely to result in injury or death, being thrown out occasionally avoids injury, for example from fire. Helmets decrease the risk of head injury, but may rarely inflict damage. Those opposed to swimming pool fences even try to argue, contrary to the evidence, that pool fences may give a false sense of security and increase the risk of drownings.

Are the above public health interventions comparable to immunization, because the benefits outweigh the risks, or is immunization different? It seems to us that there is an important difference between immunization, which involves the injection of foreign material, even though with the intention of protecting the recipient and the community, and the compulsory use of seat belts, crash helmets or pool fences. Compelling someone against their will to have an immunization could be seen as constituting a physical **assault**, whereas the other interventions are substantially less invasive.

Herd immunity and the paradox of the 'free riders'

Herd immunity is the phenomenon that, once a critical proportion of a population is immune to a particular transmissible disease, through infection or immunization, the disease can no longer circulate in the community.[12] The concept only applies to diseases such as diphtheria, measles and pertussis, which are confined to humans and transmissible person-to-person. If there is an animal reservoir, and no transmission from person-to-person, such as for tetanus or rabies, then an individual derives no benefit from the immunization of others in the community. An individual is only protected against tetanus if that individual is immunized.

The critical level of population immunization to achieve herd immunity varies from disease to disease. For Hib disease, rates fall rapidly once 85% of infants are immunized.[1,2] Measles requires approximately 95% immunization rates to stop any outbreaks.[2,3] Pertussis continues to circulate, although at much reduced intensity, even when high levels of immunization are retained; the reason is thought to be waning immunity in adults, who then infect babies. One major benefit of high rates of immunization is to protect, from diseases like whooping cough, babies too young to have been immunized (almost all whooping cough deaths are of babies under 3 months old).[7]

An important implication of herd immunity is that failure to immunize a child against a transmissible infection may not only render that child susceptible to infection, but may imperil other children. Unimmunized school children in Colorado had a greatly increased risk of catching measles (22-fold) and pertussis

(6-fold).[13] In addition, pertussis outbreaks were more likely in schools with a higher percentage of unimmunized children.[12] Immunization against pertussis is not 100% protective, so fully immunized children were catching pertussis, and possibly transmitting it to their infant siblings, yet the pertussis was circulating largely because some of their fellow school children were not immunized.

When the population is highly immunized against a disease subject to herd immunity, then a parent may elect not to have their child immunized, and the child is protected by the herd. Such parents are sometimes referred to as 'free riders'.[14] If the number of free riders increases, the population becomes more susceptible, and the disease will start to circulate.

What this description also illustrates is that the risk-benefit equation of immunization against a transmissible infection varies for any single child in the community according to community rates of immunization. If almost all other children are immunized, then a child can be unimmunized and benefit from herd immunity. If vaccine-preventable diseases like measles and pertussis are circulating, because of low levels of immunization, the benefit of immunization for any individual far outweighs the risk. A corollary is that immunization of a child against a transmissible infection protects the community as well as protecting that individual.

Arguments in favour of compulsory immunization

(i) Communitarian

Communitarianism is a modern term for a philosophical theory that insists that we recognize the value not only of individual freedom but also of the common good.[15] Although communitarianism is a modern term, it is an ancient concept: philosophers such as Aristotle and David Hume espoused the importance of the community.[15]

A communitarian may well argue that immunization benefits the whole community and protects the common good of society, and that since its significance in protecting the common good outweighs its significance in limiting individual freedom, immunization should be compulsory. An extreme communitarian might say that everyone in the community should be immunized (unless there is a medical contra-indication) and that anyone who declined immunization was effectively declining to be part of the community and should be forced to leave the community. A moderate communitarian would find a less draconian sanction for non-compliance.

(ii) Consequentialist

Consequentialists or utilitarians argue that actions or policies are good or bad according to the balance of their good and bad consequences. Compulsory immunization would be preferable to voluntary immunization if it produced the best overall result, from a perspective that gives equal weight to the interests of each affected party. Compulsory childhood immunization would almost certainly result in less disease and hence less suffering, which would outweigh vaccine adverse events. A bad consequence to consider, however, is the limitation to personal freedom occasioned by coercing people to immunize their children. If compulsory immunization caused concern about coercive government control, yet voluntary immunization could achieve almost equally high rates, then a consequentialist might prefer voluntary immunization. If compulsory immunization was the best way to protect children and was acceptable to the community, then a consequentialist should favour compulsory immunization.

(iii) Rights-based: rights of the child and the community

An advocate of children's rights may well argue that, because children need to be protected from dangerous infectious diseases, they have a right to the protection afforded by immunization.[16] Such a right, it might be argued, generates a duty on the part of parents (or, if they are negligent in the fulfilment of that duty, on the part of the state) to immunize the child. Since it is well known that some parents will be negligent with respect to this duty, the state must accept that it has the duty to ensure that each child is immunized, and if it is objected that parents have a right to decide how best to look after their offspring's health and well-being, the advocate of the child's right may well claim that the child's right to protection has priority over the parents' right to decide.

A communitarian might say that the community's interests should take preference over individual rights. How do we decide which rights should be paramount: the child's, the parents' or the community's? One answer is the degree of risk.[17] If the risk to the child or the community is high, then it may be necessary to over-ride the parents' right to choose. A child bitten by a rabid dog will almost certainly die unless given rabies vaccination. If a parent refused rabies vaccination in this circumstance, the situation would be a child protection issue, and the child's right to protection would be the paramount consideration. This situation is analogous to a child of Jehovah's Witness parents who is bleeding to death: the child is too young to choose, and the child's safety becomes pre-eminent.

If there was an outbreak of a vaccine-preventable disease, which was devastatingly severe and children could not be protected simply by exclusion from school, it might be argued that compulsory immunization would be justified. An example might be an outbreak of smallpox due to a bioterrorist attack.

Arguments against compulsory immunization

(i) Respect for parental autonomy

Respect for the autonomous choices of other persons is one of the most deep-rooted concepts in moral thinking. It is tempting for proponents of immunization to say that a child cannot make an autonomous decision about immunization and we should over-rule parents who decline to have their children immunized. But how far should we interfere with parental choices about child rearing? In any society, particularly a pluralist or multicultural society, there are many views on what is acceptable in rearing children. In general, parents have to live with their choices for their children and it is usual to respect such parental choices. The only exceptions to this are when the parents' actions or choices result in serious harm or neglect, i.e. child protection issues.

(ii) Rights-based: rights of the parents

A rights-based approach can also be used to argue against compulsory immunization, because the child's parents also have rights. These rights derive from the fact that they conceived, bore and reared the child and have a significant emotional and financial investment in the child's current and future well-being. This creates an obligation on others to respect parents' right to bring up their children as they see fit, unless they cause serious harm to the child. To argue that parents should be compelled to immunize their children in the child's 'best interest' is to ignore the fact that a child is part of a family. The child of parents who are religiously or

philosophically opposed to immunization is quite likely to grow up opposed to immunization. To have been forcibly immunized in childhood will then be viewed by the adult as a societal assault.

(iii) Variable risk-benefit of different vaccines

Even if protection of the community is a compelling communitarian argument for compulsory immunization, it only applies to transmissible infections, and not tetanus. Furthermore, the risk-benefit equation varies from disease to disease and varies over time for a single disease, depending on incidence. To make all routine childhood immunizations compulsory risks ignoring these important intrinsic differences.

(iv) Trust versus State coercion

The state already applies coercion to many of our daily activities. Do we want to live in the sort of society that extends coercion to routine immunization? At present, many industrialized countries achieve high levels of immunization without the need for compulsion. If such high levels can be maintained through encouragement and incentives, this effectively achieves the aims of the moderate communitarian, without the need for legislation. Compulsory immunization would be certain to inflame those who already believe that their Government interferes too much with their freedom. What is more, coercion may alter perception of risk. People who are coerced into an action may be more likely to perceive the action as being risky than if they are persuaded into it. Recent examples, albeit adult rather than child, have been the mandatory immunization of military personnel against anthrax and smallpox, which led to many protests and loss of confidence. Most parents trust the assurances of health care professionals that the benefits of immunizing their child outweigh the risks. Making immunizations compulsory renders trust redundant. If State coercion can be avoided in the area of routine childhood immunization, so much the better.

(v) Practical issues

Even if it was decided that routine childhood immunization should be compulsory, there are potential practical difficulties in enforcement. We often physically restrain a young child to immunize them, but with parental consent. To physically restrain a child and immunize them against their parents' wish could constitute an assault, which only seems justifiable in a situation of extreme risk, such as post-rabies exposure. The alternative is to introduce sanctions for non-compliance, such as fines or even draconian measures like child care proceedings or imprisonment.

Alternatives to compulsory immunization

Most countries do not have compulsory routine childhood immunization. Instead they employ one or more of the following strategies:

(i) Education

If education of the community and of health care providers about the benefits of immunization achieves levels of vaccine uptake that prevent circulation of infectious diseases, then it is unnecessary to introduce legislation to compel parents to conform.

(ii) Inducements

Inducements may be offered to parents or to providers, such as general practitioners. Inducements to parents usually take the form of linking child care benefit payments and/or maternity benefits to immunization status. Could this be seen as a form of coercion, particularly to poorer families who are far more dependent on such welfare payments? A communitarian might argue that if society provides child and family payments, it is reasonable for society to expect and even demand that children be immunized to help protect the whole community. A comparable situation might be taxes on cigarettes and alcohol. To ban cigarettes or alcohol infringes autonomy and is too coercive. Taxation is less coercive and is proportional (the more you smoke and drink the more you pay). Both taxation of cigarettes and financial penalties for non-immunization follow principles of distributive justice. Smoke if you must, but your taxes will off set the cost to society of smoking-related illnesses. If you choose not to immunize your child, the benefit payments saved will help pay for the cost of infectious diseases.

(iii) School exclusion during outbreaks

In New Zealand and some states of Australia, evidence of children's immunization status must be presented at school entry.[18] Immunization status rather than immunization is compulsory. Unimmunized children are excluded from school during outbreaks.

(iv) Outbreak legislation

It is possible to enact emergency legislation to compel immunization in the event of an outbreak, such as an influenza pandemic or a bioterrorist smallpox attack. On the other hand, compulsion is scarcely likely to be necessary when the threat of death is very high.

(v) No fault vaccine injury compensation schemes

If the State makes immunization compulsory, then it seems mandatory that the State should compensate the few children who are injured by vaccines. Compensation should be for medical costs, pain and suffering, disability benefits and, if necessary, benefits for loss of earning and death.[19]

It could be argued that, because parents have their children immunized in good faith, and because no-one is to blame for the rare, severe, unpredictable vaccine injuries that occur, then Governments should introduce no-fault compensation schemes even when immunization is voluntary. Thus no-fault vaccine injury compensation schemes probably ought to be in place regardless of, rather than as an alternative to, compulsory immunization laws. There are at least 13 vaccine injury compensation programmes in the world, and immunization is compulsory in only four of those countries.[19]

CONCLUSION

Compulsory immunization will be regarded by many as justifiable in terms of the benefit to the individual child and to the community. But, in order to respect autonomy, State coercion should be kept to a minimum. We believe that, in general, children should not be compulsorily immunized when similar results can be achieved by education and inducements. Australia is in the happy position of having achieved very high rates of routine childhood immunization, over 90%, without the need for compulsion.[20]

The case for compulsion might be stronger if immunization levels fell, but might not be necessary, because in that case epidemics would occur and the public would quickly recognize the value of immunization.

Whether or not childhood immunization is compulsory, a strong ethical case can be made for introducing a no-fault compensation scheme in Australia, and indeed in other countries.

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Chapter 4: Vaccine Safety

Extensive limb swelling reactions following DTPa-containing vaccines

Post licensure surveillance of adverse events is an important component of any immunisation campaign. Rare adverse events not observed in smaller clinical trials may not be apparent until a vaccine is introduced into the community. Adverse events monitored in clinical trials include local injection site reactions and systemic reactions such as fever, general malaise, headache etc.

Through passive surveillance of adverse events after immunisation in South Australia, an increase in the rate of injection site reactions (ISRs) after the fourth dose of DTPa vaccine and an increased relative risk of an ISR after DTaP primary immunisation were observed, in comparison with diphtheria, tetanus, whole cell pertussis vaccine (DTPw). Similarly, in other countries, increases in the rates and severity of ISRs were observed with successive doses of DTPa vaccine. Previous studies had indicated that the incidence ranges from 2% to 24% depending on the definition of ISR used. In contrast, rates of systemic reactions seemed to remain constant or even to decrease with later DTPa vaccine doses. The pathogenesis of ISRs is complex, probably multifactorial, and not fully understood. Various hypotheses have been formulated, which include a role of the antigen content of the vaccines, high pre-vaccination levels of antibodies, the use of aluminium adjuvants, and a Th2 orientation of cytokine production.

Of greater concern is the increased incidence of extensive limb swelling (ELS) reported after booster doses of DTPa vaccines. An ELS reaction is defined as swelling and/or redness over a substantial area, involving at least one half of the circumference of the limb and involving an adjacent joint above or below the injection site, commencing within 48 hours after immunisation and resolving completely without sequelae. This type of reaction is not unique to DTPa vaccines and has been observed after administration of other vaccines, such as DTPw and hepatitis B vaccines.

The reduced-antigen content diphtheria-tetanus acellular pertussis (Tdap) vaccine was shown to result in less-extensive ISRs when used as booster immunisations for 4 - to 6 -year-old children who were primed with DTPw.

Our unit has been involved in research into ELS reactions following booster doses of DTPa containing vaccines.

13. Jacquet JM, Begue P, Grimprel E, Reinert P, Sandbu S, Silverdal SA, Faldella G, Nolan T, Lambert S, Richmond P, **Marshall H**, Roberton D, Schuerman L. Safety and immunogenicity of a combined DTPa-IPV vaccine administered as a booster from 4 years of age: a review. *Vaccine* 2006;24:2440-2448.

This review paper examined the cumulative safety and immunogenicity data for the combination DTPaIPV vaccine discussed in Chapter 1. Although there are limitations when comparing reactogenicity data between studies and between countries, some trends can be detected across the studies reviewed in this paper. Local reactions following booster vaccination with DTPa–IPV appear more frequent after primary vaccination with an acellular pertussis vaccine than after whole-cell pertussis priming. These observations are in line with the literature, which describes redness ≥50mm in 15.2–50% and swelling ≥50mm in 15.8–48.1% of children aged 4–6 years after a 4th or 5th dose of DTPa or DTPa–IPV vaccine.

Large injection site swelling reactions were reported after DTPa–IPV was given either as the fourth or fifth dose of DTPa. When given a fourth dose, 3.3% of vaccinees reported diffuse swelling (extending beyond the immediate vicinity of the injection site) but with no extension to an adjacent joint. When given as a fifth dose, diffuse swelling was more frequent (6.5%), with some further extension observed. However only 1.2% of the vaccinees reported swelling that involved the elbow joint. This frequency is similar to that reported after vaccination with DTPa vaccine in comparative studies in this age group. These results also confirmed previous findings with the same DTPa–IPV vaccine, and are consistent with literature reports of entire upper arm (shoulder to elbow) swelling in 2.0–2.9% of recipients of a fifth DTPa dose.

Despite this phenomenon of increased local reactions with repeated doses, a fourth or fifth consecutive dose of an acellular pertussis-containing vaccine is still less reactogenic than a DTPw fourth or fifth booster dose. Indeed, published studies have shown incidences of any pain, redness and swelling ranging from 28.9 to 100% after a fourth or fifth consecutive DTPw dose, with grade 3 local symptoms ranging from 10.0 to 52.6%.

A DTPa booster after a DTPw primary vaccination appears to be less reactogenic, with a DTPa primary and booster series leading to an intermediate frequency of reactions.

In addition, on review of all the previous studies, DTPa–IPV vaccine was overall highly immunogenic and elicited a satisfactory immune response, whether whole cell or acellular pertussis vaccines were administered for primary vaccination.

14. **Marshall H,** Gold M, Gent R, Quinn P, Piotto L, Clarke M, Roberton D. Ultrasound Examination of Extensive Limb Swelling Reactions After Diphtheria-Tetanus-Acellular Pertussis or Reduced-Antigen Content Diphtheria-Tetanus-Acellular Pertussis Immunization in Preschool-Aged Children. Pediatrics. 2006;118(4):1501-1509.

An extensive limb swelling (ELS) reaction after booster doses of the acellular pertussis combination vaccines can be alarming to parents and vaccine providers. If the nature of the reaction is not recognised, an incorrect diagnosis of infective cellulitis may be made, resulting in inappropriate treatment with antibiotics and often resulting in inappropriate hospitalisation. Despite the observed increase reactogenicity these extensive swelling reactions do not cause significant disability as children are often only mildly limited in their activities despite the increase in upper limb redness and swelling. In particular, pain does not appear to be a prominent feature in children with ELS reactions despite significant redness and swelling.

In this paper we describe a clinical trial comparing DTPa versus dTpa (lower antigen diphtheria, tetanus pertussis vaccine) in children who had previously developed an ELS reaction following the 18 month booster DTPa immunisation. The aim of this study was to describe and compare the ELS reactions following DTPa vs dTpa vaccine by use of clinical and ultrasound assessment.

During this study the maximal swelling, redness, and induration of the affected upper arm were measured in millimetres by parents/caregivers, following vaccination. The child was examined by a study medical officer within 24 hours after notification of swelling by the parent/guardian. Ultrasound examinations were performed to examine and compare the extent of swelling in children receiving DTPa or dTpa at 4 years of age. Clinical and ultrasound examination of the ELS reaction were conducted 24 to 48 hours after immunisation and repeated 48 to 96 hours after the first ultrasound assessment. The ultrasound assessments were blinded with respect to use of DTPa or dTpa vaccine. Subcutaneous tissue thickness and muscle thickness were measured in both the affected arm and the non affected arm, and the absolute values were compared. Ultrasound examination of the joint was performed for children with clinically apparent swelling extending to a joint.

Ultrasound examinations showed a diffuse, echogenic, "snowstorm" appearance, consistent with diffuse oedema of the tissues. All children showed evidence of oedema in both subcutaneous and muscle tissue, extending to the humeral cortex. Subcutaneous and muscle tissues expanded to a maximum of 281% and 111% of the tissue thickness of the control arm, respectively. No fluid was detected in the shoulder joint for children who clinically exhibited swelling that extended over the shoulder joint. All children developed swelling of subcutaneous and muscle tissues.

The mean percentage increase in swelling of subcutaneous tissue for children who received DTPa vaccine was 136.0% (95% confidence interval [CI]: 73.1%–198.0%), compared with 124.3% (95% CI: 66.5%–182.0%) for children who received dTpa vaccine. This descriptive study formed part of a larger study (to be submitted for publication). Although the sample size was too small to determine statistical significance of the results, the larger study showed a trend with reduced reactogenicity in the group receiving the lower antigen content vaccine.

This study demonstrated that the ELS reactions are attributable to marked oedema in both the subcutaneous and muscle tissue spaces, with fluid accumulation being greater in the subcutaneous tissue space. It is interesting to note that there was significant subcutaneous oedema, given that the vaccines were administered by the intramuscular route. The swelling of muscle tissue was generally not as extensive and seemed to resolve more rapidly than the swelling in subcutaneous tissue; this might be related to the better blood supply to muscle, compared with subcutaneous tissue.

I presented these study results at the 9th National Immunisation Conference/1st Asia Pacific Vaccine Preventable Diseases Conference, in Cairns, Queensland, Australia, August 19 – 20, 2004.

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Safety and immunogenicity of a combined DTPa–IPV vaccine administered as a booster from 4 years of age: A review

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Abstract

A combined DTPa–IPV booster vaccine was administered as a 4th or 5th dose after DTPa or DTPw priming. Over 99% vaccines developed antibody levels considered to be protective to diphtheria, tetanus and poliovirus, and >95% mounted a response to acellular pertussis antigens. Rectal temperature >39.5 °C was observed in at most 3.2% of vaccinees. Swelling >50 mm occurred in 24% of DTPa-primed compared to 5.5% of DTPw-primed children. Large swelling involving the entire upper arm (extending to involve the elbow joint) was reported for up to 1.2% of DTPa-primed subjects, which is consistent with literature reports for other DTPa vaccines.

Keywords: Immunogenicity; Vaccine-associated adverse events; Acellular pertussis vaccine; Booster vaccination

1. Introduction

In many industrialised countries, a change in the epidemiological pattern of pertussis has been witnessed in the aftermath of high DTP (diphtheria–tetanus–pertussis) vaccination coverage. In the pre-vaccine era, the peak incidence of whooping cough was in children aged 1–5 years [1], who constituted the main reservoir for pertussis infection. Natural immunity following infection was maintained into and throughout adulthood by re-exposure to circulating *Bordetella pertussis*. Today, after the major decline in the overall incidence of pertussis that followed the widespread introduction of DTP vaccination in the 1940s, a resurgence of the disease is being observed. This is characterised by a switch in the incidence to older age groups, from school-age children to adolescents and adults, as well as to young partially vaccinated infants [2–11]. This resurgence, observed in countries with a sustained and widespread use of pertussis vaccine,

Abbreviations: DTP, diphtheria–tetanus–pertussis combined vaccine; DTPa, diphtheria–tetanus–acellular pertussis combined vaccine; DTPw, diphtheria–tetanus–whole-cell pertussis combined vaccine; GMC, geometric mean concentrations; GMT, geometric mean titres; IPV, inactivated polio vaccine; OPV, oral polio vaccine; MMR, measles–mumps–rubella

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is the consequence of waning of immunity following vaccination or disease and the lack of regular natural or vaccine boosters.

All the observations suggest that the mere reinforcement of vaccination coverage within the first 2 years of life is insufficient to stop the circulation of *B. pertussis*, and will not prevent transmission to the youngest infants. Strategies to protect this vulnerable population are being devised. These include neonatal immunisation against pertussis [12], immunisation of adults including women planning pregnancy, and immunisation of adolescents in order to prevent transmission to young infants [13]. Still, there is a clear need for booster doses of pertussis vaccine beyond infancy in order to prolong immunity. Based on local epidemiological features and other considerations, recommendations for a late pertussis booster involving pre-school or pre-adolescent [14] children are appropriate.

Existing primary vaccination schedules vary widely; the DTP booster may be given not only at different ages, but after a pertussis primary series differing in nature (whole-cell or acellular) and in the number of doses given. The purpose of this article is to review the clinical experience with GlaxoSmithKline Biologicals' combined DTPa–IPV vaccine when administered as a late booster in representative situations: after DTPa or DTPw primary vaccination, at 4–7 years of age or at 11–13 years of age, as a 4th or 5th dose of pertussis vaccine, or after primary vaccination with an oral or inactivated polio vaccine (OPV and IPV, respectively). The review will evaluate the immunogenicity and the safety of the vaccine.

2. Methods

All clinical studies conducted by GlaxoSmithKline (GSK) Biologicals with DTPa–IPV given in early childhood after a primary immunisation series are presented. The six studies, performed in five countries, served as the basis for the regulatory approval of the late booster indication for the vaccine. The study protocols were approved by the relevant ethics committees and the studies were conducted according to the Declaration of Helsinki and Good Clinical Practice Guidelines. Written informed consent was obtained from the parents or guardians of all children before their enrolment. Routine vaccination had been administered previously according to the standard schedule recommendations in the respective countries. The reactogenicity and safety of the vaccine were evaluated in the six studies, and its immunogenicity was evaluated in five of them.

2.1. Study design

The design of the studies presented here is summarised in Table 1. All studies were conducted in an open fashion. Four studies were randomised so as to include a comparator group receiving DT–IPV (studies B and E) or separate injections of DTPa and IPV vaccines (studies C and D). In study D, the second dose of measles–mumps–rubella (MMR) vaccine was administered concomitantly with DTPa–IPV or DTPa and IPV. A history of a severe adverse reaction possibly related to the pertussis vaccine was an exclusion criterion at study entry, as classically recommended in the prescribing information of pertussis-containing vaccines. After physical examination and recording of body temperature, vaccines were given by injection in the deltoid muscle. Each subject was monitored for at least 15 min after the injection.

2.2. Vaccines

Each 0.5 ml dose of the DTPa–IPV vaccine (*Infanrix*™-IPV, GSK Biologicals) contains three acellular pertussis antigens (25 μg of pertussis toxoid (PT), 25 μg of filamentous agglutinin (FHA), 8 μg of pertactin (PRN)), ≥30 IU of diphtheria toxoid, ≥40 IU of tetanus toxoid, 40 D-D antigen units of type 1 poliovirus (Mahoney strain), 8 D-D antigen units of type 2 poliovirus (MEF-1 strain) and 32 D-D antigen units of type 3 poliovirus (Saukett strain).

2.3. Reactogenicity assessment

On the day of vaccination and the following 3 days, parents completed diary cards soliciting local and systemic symptoms. These were graded in intensity as 1 (easily tolerated),

Table 1
Vaccination history and design of studies

Study	*N*	Country	Previous vaccines and schedule	Booster (DTPa–IPV)[a]	
				Age	Comparator
A	128	Norway	DTPw and IPV at 3, 5, 10–12 months	6–7	None
B	73	France	DTPw and IPV at 2, 3, 4 and 16–18 months	5–6	DT–IPV
C [25]	210	Sweden	DTPa and IPV at 3, 5, 10–12 months	4–6	DTPa and IPV
		Italy	DTPa at 3, 5, 10–12 months; IPV at 3, 5 months, OPV at 10–12 months and at 3 years	4–6	DTPa and IPV
D[b] [27]	181	Australia	DTPa at 2, 4, 6 and 12–24 months; IPV (or OPV) at 2, 4, 6 months	4–6	DTPa and IPV
E [26]	60	France	DTPw and IPV at 2, 3, 4 and 16–18 months; DT–IPV at 5–6 years	11–13	DT–IPV
F	641	France	DTPw and IPV at 2, 3, 4 and 16–18 months; DT–IPV at 5–6 years	11–13	None

[a] In all studies, subjects received the vaccines described in column 4, but in the booster trials described here, subjects received either combined DTPa–IPV or the comparator listed.

[b] In study D, the MMR vaccine was concomitantly administered with DTPa–IPV and with DTPa and IPV. *N* = number of DTPa–IPV vaccinees.

2 (interfering with daily activities) or 3 (preventing normal daily activities). Redness and swelling at the site of injection were measured and graded as >20 and >50 mm. Fever was defined as rectal temperature ≥38.0 °C and grade 3 fever as rectal temperature >39.5 °C. Space was also provided on diary cards to record any other symptom occurring during the 30 days following vaccination.

An increase in local reactogenicity, particularly in terms of swelling, has been reported for the 4th and 5th consecutive doses of DTPa vaccines [15–19]. In studies C and D, where children received a full vaccination series with an acellular pertussis vaccine, large swelling reactions of the injected limb following vaccination were to be reported by the parents. These were analysed according to the following three categories: (i) local swelling reactions, i.e. confined to the injection site, with diameter >50 mm; (ii) diffuse swelling reactions not involving the adjacent joint; (iii) diffuse swelling reactions involving the adjacent joint. Associated functional impairment was recorded and scored.

2.4. Serological analyses

Blood samples were collected before and 1 month after the administration of study vaccines in all studies except study F. Sera were kept at −20 °C until serological analysis at GSK Biologicals for studies B, C, D and E. Serological assays for study A were performed at the laboratory of Dr. Michael Pichichero (University of Rochester, USA). Anti-diphtheria and anti-tetanus antibodies were measured by an enzyme-linked immunosorbent assay with a cut-off of 0.1 IU/ml, which is a conservative estimate for protective antibody concentrations [20,21]. Neutralising antibodies to diphtheria were also measured in study C using an in vitro neutralisation assay on Vero cells [22,23] with a cut-off of 0.016 IU/ml. Antibodies against the three pertussis antigens were determined by enzyme-linked immunosorbent assay with cut-off, for all three pertussis antigens, set at 5 EL U/ml, which was three to four times higher than the lower quantitation limit of the assay. Also evaluated were the sero-responses to PT, FHA and PRN, defined as the appearance of antibodies in initially seronegative children and at least a two-fold increase in antibody concentration in initially seropositive children. Neutralising antibodies to poliovirus types 1, 2 and 3 were determined using a modification of the WHO/EPI microneutralisation test method [24], a titre of 8 being considered as seroprotective.

2.5. Statistical analysis

All analyses were performed using SAS software. In comparative studies, between-group analysis was performed using Student's *t*-test for post-vaccination antibody geometric mean concentrations/titres (GMC and GMT, respectively), and using two-sided Fisher's exact test for incidences of symptoms. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Immunogenicity

A total of 619 subjects in five clinical studies were evaluated for the immune response to the DTPa–IPV vaccine. One month after the DTPa–IPV booster dose, all subjects had seroprotective levels of antibodies against diphtheria and tetanus toxoid and poliovirus, with the exception of one DTPa–IPV recipient, who still had anti-polio 3 titre <8 after booster vaccination in study C [25]. In children 4–7 years of age (studies A–D), more than 95% of the study subjects showed a seroresponse (appearance of detectable levels of antibodies or at least a two-fold rise in antibody levels) to the three pertussis antigens. In the age group 11–13 years, all subjects developed a sero-response to FHA and PRN and 66% to PT (study E [26]).

GMCs of antibodies to each antigen contained in the vaccine are shown in Table 2. In comparative studies, some differences in GMCs were noted with the comparator vaccines. GMCs of antibodies against all pertussis antigens were similar pre- and post-booster in the DT–IPV groups (studies B and E) who received no pertussis antigens. GMCs of anti-diphtheria and anti-tetanus antibodies were significantly higher in the DT–IPV control group in studies B and E. The GMTs of antibodies against poliovirus were significantly higher after DTPa–IPV than after DT–IPV (for type 3 in study B and types 1, 2, and 3 in study E); whereas GMTs of antibodies against poliovirus types were significantly higher in the DTPa + IPV control group (for types 1, 2 and 3 in study C, and type 2 in study D).

In study D, where a 2nd MMR vaccine dose was given concomitantly, all the subjects who were seronegative for measles, mumps or rubella prior to vaccination were seropositive afterwards, with the exception of one subject, receiving DTPa and IPV separately, who did not respond to measles [27].

3.2. Reactogenicity

A total of 1293 subjects were evaluated for safety and reactogenicity after the administration of the DTPa–IPV booster in six clinical studies. The incidences of fever were similar in the four studies conducted in children 4–7 years of age (Table 3). Fever above 39.5 °C was infrequently observed. The highest incidence (3.2%) was seen in study A. Fever was less frequently reported (0.2%) in the 11–13 years age group (studies E and F) than in younger children.

Pain was the most frequently reported symptom in all studies and groups. It is to be noted that in studies C and D, where local reactogenicity is reported regardless of the vaccination site for separate administration control groups, the DTPa vaccine is the main contributor to the incidence of local reactions in these groups. The DTPa–IPV vaccine was not more reactogenic than separately co-administered DTPa and IPV (studies C and D, Tables 3 and 4). It led however, to significantly

Table 2
Geometric mean antibody concentrations and titres with 95% CI

Vaccine	*N*	Anti-D (IU/ml)	Anti-T (IU/ml)	Anti-PT (EL U/ml)	Anti-FHA (EL U/ml)	Anti-PRN (EL U/ml)	Anti-polio 1	Anti-polio 2	Anti-polio 3
Study A									
DTPa–IPV									
Pre	119	0.1 (0.08–0.11)	0.8 (0.66–0.96)	3.1 (2.7–3.5)	14.4 (11.8–17.7)	13.7 (11.2–16.6)	73.5 (63.2–85.4)	91.9 (78.4–107.8)	75.1 (59.8–84.4)
Post	119	5.8 (4.8–6.9)	18.7 (16.6–21.0)	52.1 (43.8–61.9)	318.1 (279.3–362.4)	407.3 (330.8–501.5)	1423 (1229–1648)	1614 (1393–1871)	1823 (1506–2206)
Study B									
DTPa–IPV									
Pre	72	0.1 (0.09–0.15)	0.3 (0.2–0.32)	3.6 (3.0–4.3)	31.8 (22.1–45.9)	16.8 (12.7–22.3)	15.6 (11.7–20.8)	21.8 (16.3–29.0)	44.4 (31.9–61.7)
Post	73	6.2* (4.8–7.9)	13.6* (11.3–16.3)	84.7* (62.5 –114.9)	1051.1*(898.3 –1229.8)	820.1*(656.8–1024.0)	1533 (1157–2032)	1053 (820–1354)	1741* (1316–2303)
DT–IPV									
Pre	71	0.1 (0.08–0.14)	0.2 (0.15–0.24)	3.4 (2.9–4.0)	26.8 (19.3–37.2)	12.3 (9.0–16.9)	18.0 (12.8–25.4)	25.7 (18.7–35.3)	62.1 (43.9–88.0)
Post	69	11.3 (8.9–14.5)	19.3 (15.5–24.0)	3.5 (3.0–4.0)	26.1 (19.3–35.2)	11.7 (8.5–16.1)	1461 (1104–1933)	1012 (811–1264)	982 (774–1246)
Study C									
DTPa–IPV									
Pre	201	0.08 (0.07–0.09)	0.15 (0.13–0.18)	3.6 (3.2–4.0)	30.0 (24.9–36.2)	27.2 (23.0–32.3)	65.3 (49.9–85.4)	41.4 (32.0–53.5)	23.5 (19.3–28.7)
Post	208	6.2 (5.4–7.2)	10.0* (8.8–11.3)	63.2* (56.1–71.2)	735.2 (653.4–827.4)	995.6 (863.5–1147.9)	2096* (1818–2417)	1702* (1482–1955)	2543* (2122–3047)
DTPa + IPV									
Pre	189	0.09 (0.08–0.1)	0.2 (0.15–0.19)	3.6 (3.3–4.0)	31.8 (26.9–37.8)	23.9 (20.1–28.4)	56.7 (44.2–72.6)	45.9 (36.4–58.0)	22.9 (18.6–28.1)
Post	195	6.2 (5.4–7.2)	8.5 (7.7–9.4)	77.9 (68.3–88.8)	830.0 (747.9–921.2)	1021.3 (904.2–1153.6)	2764 (2429–3145)	2736 (2400–3118)	3274 (2820–3802)
Study D									
DTPa–IPV									
Pre	160	0.19 (0.15–0.23)	0.36 (0.30–0.42)	5.4 (4.7–6.3)	35.3 (30.0–41.7)	39.6 (33.9–46.2)	53.9 (42.0–69.2)	82.6 (64.5–105.7)	42.9 (34.5–53.5)
Post	166	5.9 (5.1–6.9)	7.9* (7.1–8.7)	110.9 (94.7–129.8)	372.0 (330.8–417.3)	707.0 (619.2–807.4)	3014 (2591–3507)	2883*(2484–3346)	4849 (4258–5521)
DTPa + IPV									
Pre	154	0.21 (0.17–0.26)	0.41 (0.35–0.48)	5.4 (4.7–6.3)	28.6 (24.4–33.5)	38.2 (32.4–45.2)	45.6 (35.3–58.8)	91.1 (70.4–117.8)	47.2 (37.4–59.5)
Post	163	6.0 (5.1–7.0)	6.8 (6.2–7.4)	127.9 (112.4–145–5)	409.6 (362.9–462.3)	663.0 (575.8–763.5)	3219 (2758–3757)	3532 (3075–4056)	4865 (4296–5510)
Study E									
DTPa–IPV									
Pre	53	1.0 (0.8–1.3)	1.9 (1.5–2.4)	93.2 (83.4–104.2)	30.8 (23.2–40.9)	23.9 (17.9–31.9)	182.2 (126.9–261.6)	105.2 (77.3–143.2)	85.4 (62.8–116.1)
Post	53	11.3* (8.9–14.3)	10.1* (8.8–11.6)	305.4* (246.8–377.8)	795.0* (630.9–1001.8)	708.6* (538.6–932.1)	1374 (1015–1862)	1093* (804–1487)	2714* (1975–3730)
DT–IPV									
Pre	49	1.0 (0.8–1.4)	1.8 (1.4–2.4)	96.1 (84.3–109.5)	32.7 (24.3–44.1)	20.6 (16.2–21.6)	220.8 (155.6–313.5)	121.8 (89.2–166.3)	97.9 (68.8–139.3)
Post	49	21.9 (16.2–29.6)	24.8 (19.9–30.8)	96.6 (85.6–109.1)	39.4 (30.9–50.2)	21.4 (16.9–27.2)	1673.1 (1238–2262)	709.9 (526–942)	995.2 (708–1398)

* Significant difference as compared to control group ($p < 0.05$ two-sided Student's *t*-test), comparison only performed for post-vaccination time point.

Table 3
Percentage of children reporting symptoms during the 4 days following booster vaccination

Study	Previous vaccines	Study vaccine	N	Temperature		Pain		Redness			Swelling		
				≥38.0 °C	>39.5 °C	Any	Grade 3	Any	>20 mm	>50 mm	Any	>20 mm	>50 mm
Booster at 4–7 years of age													
A	3DTPw	DTPa–IPV	124	18.5	3.2	80.6	6.5	21.8	8.1	1.6	19.4	9.7	1.6
B	4DTPw	DTPa–IPV	73	15.1	0.0	82.2*	5.5	65.8	54.8*	11.0*	52.1*	32.9	5.5*
		DT–IPV	71	14.1	0	64.8	8.5	52.1	26.8	0	23.9	11.3	0
C[a]	3DTPa	DTPa–IPV	210	21.0	0.8	71.4	2.9	61.0	39.0	25.7	53.3	28.6	13.3
		DTPa + IPV	201	16.8	1.5	69.2	5.0	61.7	47.3	31.8	54.2	33.3	16.4
D[a]	4DTPa	DTPa–IPV + MMR	171	22.8	1.8	81.3	7.0	80.7*	60.2	32.7	62.0*	43.9	24.0
		DTPa + IPV + MMR	167	26.9	0.6	75.4	4.8	89.2	68.9	41.9	75.4	44.3	28.1
Booster at 11–13 years of age													
E	4DTPw	DTPa–IPV	59	5.1	0.0	84.7	10.2	49.2	23.7	6.7	54.2	11.9	0.0
		DT–IPV	53	9.4	0.0	81.1	5.7	60.4	30.2	7.3	49.1	9.4	3.6
F	4DTPw	DTPa–IPV	641	12.7	0.2	80.0	6.4	40.9	18.1	2.3	37.7	13.3	1.4

[a] In studies C and D, local reactions are reported for any site, i.e. reactions noted at the sites of injection of the MMR and IPV vaccines are taken into account.
* Significant difference as compared to control group (two-sided Fisher's exact test p-value < 0.05).

($p < 0.05$) more local reactions than the DT–IPV comparator in 4–7-year-old children (study B). Such a difference between the two vaccines was not seen in 11–13-year-olds in study E.

Redness and swelling of more than 50 mm in diameter appeared more frequently after DTPa priming (studies C and D) than after DTPw priming. Redness and swelling >50 mm were reported for 1.6–11.0% of vaccinees in studies A, B, E and F, primed with DTPw, and in 13.3–32.7% of the vaccinees in studies C and D, primed with DTPa. Large injection site swelling reactions were solicited in studies C and D after a full DTPa vaccination series (Table 4). In both studies, the large majority of these reactions were limited to the vicinity of the injection area. Diffuse swellings with no involvement of an adjacent joint were observed in 3.3% of DTPa–IPV vaccinees in study C (4th DTPa dose), and in 0.6% of DTPa–IPV vaccinees in study D (5th DTPa dose). In the latter study, a total of 11 subjects (6.5% of DTPa–IPV vaccinees) experienced diffuse swelling. For eight of those (4.7% of DTPa–IPV vaccinees) swelling involved the shoulder area, immediately adjacent to the site of injection, and two subjects (1.2% of DTPa–IPV vaccinees) recorded swellings of the whole upper arm (from shoulder to elbow), involving the elbow joint. Swelling of the whole arm down to the wrist was not reported. These swelling reactions typically occurred within 2 days of vaccination, and completely resolved after an average of 4 days. Fewer than 10% of the observed large injection site swellings (2 out of 28 in study C and 4 out of 42 in study D) were accompanied with moderate or severe functional impairment.

Table 4
Number (percentage) of subjects reporting large injection site swelling

	Study C		Study D	
	DTPa–IPV ($N = 210$), n (%)	DTPa ($N = 201$), n (%)	DTPa–IPV ($N = 171$), n (%)	DTPa ($N = 167$), n (%)
Any large swelling reaction	28 (13.3)	35 (17.4)	42 (24.6)	49 (29.3)
Local swelling confined to the injection site	21 (10.0)	27 (13.4)	31 (18.1)	30 (18.0)
Diffuse swelling not involving an adjacent joint	7 (3.3)	5 (2.5)	1 (0.6)	9 (5.4)
Diffuse swelling involving the shoulder only	0	3 (1.5)	8 (4.7)	8 (4.8)
Swelling involving the shoulder and elbow joint	0	0	2 (1.2)	2 (1.2)
Associated symptoms				
Functional impairment				
Mild	6 (2.9)	5 (2.5)	9 (5.3)	10 (6.0)
Moderate	2 (1.0)	4 (2.0)	2 (1.2)	4 (2.4)
Severe	0	1 (0.5)	2 (1.2)	0
Pain	14 (6.7)	14 (7.0)	27 (15.8)	27 (16.2)
Induration	3 (1.4)	7 (3.5)	16 (9.4)	21 (12.6)
Mean arm circumference increase[a] (cm)	2.2	2.1	2.5	2.5
Median resolution time (days)	4	3	2	3

Functional impairment—mild: easily tolerated, causing minimal discomfort and did not interfere with everyday activities; moderate: sufficiently discomforting to interfere with normal everyday activities; severe: prevented normal everyday activities.
[a] Difference in circumference between the injected arm and the opposite arm.

Two serious adverse events were reported following DTPa–IPV vaccination in study F, the safety study conducted in 11–13-year-olds. The first was a case of appendicitis in a 12-year-old, 22 days after DTPa–IPV booster vaccination. The event was judged as unrelated to vaccination. The second was a report of abdominal pain and diarrhoea, which started on the day following vaccination in a 12-year-old. The event was judged as probably unrelated to vaccination. The two subjects fully recovered. No serious adverse events were reported in the other studies.

4. Discussion

A total of 619 subjects, enrolled in five clinical studies, were evaluated for the immune response to the DTPa–IPV vaccine. The DTPa–IPV vaccine was highly immunogenic in all circumstances (Table 2). One month after vaccination, all subjects had protective levels of antibodies against diphtheria, tetanus and polio antigens, with the exception of one subject who did not achieve anti-polio 3 titre above 8 in study C. Some significant differences compared with the control group were noted for GMCs of antibodies directed against these antigens. As virtually all subjects had high and protective antibody levels for these antigens, these differences are likely of limited clinical significance.

Sero-response rates to the three pertussis antigens were high, above 95%, in all five studies. An exception was the 66% sero-response to pertussis toxoid seen in 11–13-year-olds in study E. This low percentage is likely to be explained by the fact that all subjects were already seropositive in terms of anti-PT antibodies before booster vaccination, with high antibody levels (GMC = 93.2 EL U/ml). It can be hypothesised that these high levels of anti-pertussis antibodies in this older age group were due to natural boosting via exposure to circulating *B. pertussis* [7,28,29]. Also, despite a low sero-response rate, the pre-adolescents in this study had very high anti-PT antibody GMCs 1 month after booster vaccination; more than three-fold higher than the 5–7-year-olds in study B, which was also conducted in French children [26].

Likewise, 11–13-year-olds in study E mounted higher levels of antibodies against diphtheria than younger children. It is likely due to the DTPa–IPV vaccine being a 6th dose of D, T and IPV antigens in this study, and to the fact that antibody levels against these antigens tended to be higher before the 6th dose in this study than before the 4th or 5th dose in younger children in studies A–D.

Vaccination history did not significantly impact on the immune response, either in terms of seroprotection rate or of sero-response rate for pertussis, or in terms of antibody levels against diphtheria, tetanus or polio types. Indeed, no differences in the response to the booster vaccine were noted across the four studies in 4–7-year-olds, i.e. according to number of previous DTP doses (3 or 4) received.

The DTPa–IPV vaccine was overall highly immunogenic and elicited a satisfactory immune response, whether whole-cell or acellular pertussis vaccines were administered for primary vaccination.

DTPa vaccines with a lower antigen content (dTpa and dTpa–IPV) have been developed for vaccination of adults or booster vaccination [30]. These vaccines can provide in addition reduced reactogenicity. In the pre-school age indication, these vaccines indeed lead to satisfactory levels of seroprotection and sero-response, but are however associated, at least for some antigens, with a trend to lower antibody levels than full-antigen-content DTPa vaccines. These differences in antibody levels tend to disappear over time, and their clinical relevance is not entirely clear [31,32].

Study D, which documented the co-administration of the DTPa–IPV vaccine with a second dose of the MMR vaccine, showed that this co-administration did not impair the response to the diphtheria, tetanus, acellular pertussis and polio antigens. The response to the MMR vaccine was also satisfactory, with all subjects being seropositive to the three components 1 month after vaccination [27].

A total of 1293 subjects were evaluated for safety and reactogenicity after the administration of the DTPa–IPV booster in six studies. The vaccine showed a good systemic tolerability. In subjects with a comparable vaccination history, the reactogenicity profile differed according to the age of booster vaccination; with lower incidences in older children, when the interval between the last two injections of pertussis vaccine was larger (studies E and F compared with study B). The incidence of fever was low after DTPa–IPV booster vaccination in all the studies reviewed, and rectal temperature >39.5 °C was reported infrequently.

An increased local reactogenicity has been reported after 4th and 5th consecutive doses of acellular pertussis DTP combinations [15–18]. Although there are limitations when comparing reactogenicity data between studies and between countries, some trends can be detected across the studies reviewed here. Expectedly, local reactions following booster vaccination with DTPa–IPV appear more frequent after primary vaccination with an acellular pertussis vaccine (studies C and D) than after whole-cell pertussis priming (studies A and B). These observations are in line with the literature, which describes redness ≥50 mm in 15.2–50% and swelling ≥50 mm in 15.8–48.1% of children aged 4–6 years after a 4th or 5th dose of DTPa or DTPa–IPV vaccine [15,18,33–36].

Extensive swelling reactions are recognised to occur after booster vaccination with different vaccines including DTPw [37], acellular pertussis alone [38], DTPa [15–17] and other vaccines [39]. The aetiology of these reactions is not fully understood and is still a field for research. Various hypotheses have been formulated, which include a role of the antigen content of the vaccines, high pre-vaccination levels of antibodies, the use of aluminium

adjuvants, and a Th2 orientation of cytokine production [40,41].

Large injection site swelling reactions were reported after DTPa–IPV given either as the 4th or 5th dose of DTPa. A trend for more extensive swelling reactions was seen after the 5th dose. When given a 4th dose (study C), 3.3% of vaccinees reported diffuse swelling (extending beyond the immediate vicinity of the injection site) but with no extension to an adjacent joint. When given as a 5th dose (study D), diffuse swelling was more frequent (6.5%), with some further extension observed. However only 1.2% of the vaccines reported swelling that involved the elbow joint. This frequency is similar to that reported after vaccination with the parent DTPa vaccine in comparative study D and in published studies in this age group [15,17,18,42]. These results also confirm previous findings with the same DTPa–IPV vaccine [35,43], and are consistent with literature reports of entire upper arm (shoulder to elbow) swelling in 2.0–2.9% of recipients of a 5th DTPa dose [36,40,44].

Despite this phenomenon of increased local reactions with repeated doses, a 4th or 5th consecutive dose of an acellular pertussis-containing vaccine is still less reactogenic than a DTPw 4th or 5th dose booster. Indeed, published studies have shown incidences of any pain, redness and swelling ranging from 28.9 to 100% after a 4th or 5th consecutive DTPw dose, with grade 3 local symptoms ranging from 10 to 52.6% [45,15,17]. If reactogenicity undoubtedly increases with the number of doses in all instances, it appears that a full DTPw series (primary and booster vaccinations) shows the most reactogenic boosters. The less reactogenic booster seems to be a DTPa booster after DTPw primary vaccination, with a DTPa series (primary and booster) leading to an intermediate frequency of reactions.

Two direct comparative studies [33,34] showed that moderate to severe limitation of limb movement, tenderness (moderate to severe) and fever were more common after a whole-cell vaccination series than after an acellular series. These data reveal different reactogenicity profiles for the two types of vaccines suggesting, according to the authors, an important role for inflammatory mediators in the development of reactions to the DTPw vaccine [33].

Reduced-antigen-content dTpa vaccines were developed for their potential to reduce reactogenicity, more particularly in the context of repeated DTPa vaccination [31,46], Available comparative data show that the reduction is not always significant. Indeed, limited reduction in local reactions was observed in children or adolescents previously vaccinated with DTPw vaccines [43,46,47]. Moreover, although dTpa administered as a 5th DTPa dose to pre-school children [31] reduced the frequency of redness >50 mm by half (13%) as compared with DTPa (23%), reports of swelling >50 mm were not markedly decreased (observed after 13% versus 16% of the doses, respectively). Further studies are warranted to assist in determining the appropriate age for switching to reduced-antigen-content vaccines, i.e. to define when the benefits of a reduced reactogenicity clearly outweigh a potentially lower immunogenicity.

Study D showed that the co-administration of the MMR vaccine with DTPa–IPV did not significantly increase fever, or the global incidence of local symptoms. Some reactions usually associated with MMR vaccination, such as rash, were reported in the two study groups in proportions similar to previous reports after a second MMR dose [27].

Overall the DTPa–IPV vaccine was immunogenic and led to high seroprotection rates when given as a booster to pre-school children or to pre-adolescents. The differences in response seen between children and pre-adolescents are likely explained by the pre-existing immune status. The reactogenicity of the vaccine was acceptable in all the situations envisaged, and large swelling reactions were observed with a frequency consistent with literature reports. The DTPa–IPV vaccine will fit adequately as a 4th or 5th DTP dose in a wide variety of vaccination schedules.

Within the context of recent pertussis outbreaks in school-age children, this vaccine would ensure the protection of this age group. Moreover, in addition to the direct protection provided to the vaccinees, one could expect such a boosting schedule to have a wider impact on the overall incidence of whooping cough, in particular by reducing the circulation of *B. pertussis* in the population and by the reduction of secondary transmission of pertussis to vulnerable young infants.

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Ultrasound Examination of Extensive Limb Swelling Reactions After Diphtheria-Tetanus-Acellular Pertussis or Reduced-Antigen Content Diphtheria-Tetanus-Acellular Pertussis Immunization in Preschool-Aged Children

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ARTICLE

Ultrasound Examination of Extensive Limb Swelling Reactions After Diphtheria-Tetanus-Acellular Pertussis or Reduced-Antigen Content Diphtheria-Tetanus-Acellular Pertussis Immunization in Preschool-Aged Children

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ABSTRACT

OBJECTIVE. The aim of this study was to determine the site, extent, and resolution of tissue involvement when extensive limb swelling occurred in the injected limb for children who received diphtheria-tetanus-acellular pertussis or reduced-antigen content diphtheria-tetanus-acellular pertussis vaccine at 4 to 6 years of age.

METHODS. Children who had experienced an injection site reaction at 18 months of age were assigned randomly to receive an intramuscular injection of either reduced-antigen content diphtheria-tetanus-acellular pertussis vaccine or diphtheria-tetanus-acellular pertussis vaccine between 4 and 6 years of age. Children who developed extensive limb swelling were recruited for assessment by clinical examination; ultrasound studies of the affected and opposite (control) arms were performed 24 to 48 hours after immunization and 48 to 96 hours later.

RESULTS. Twelve children with extensive limb swelling were enrolled in the study. Ultrasound examinations demonstrated swelling of both the subcutaneous and muscle layers of the vaccinated arm. Ultrasound assessment showed that the swelling exceeded the clinical measurements of skin redness and swelling. Subcutaneous and muscle tissues expanded to 281% and 111% of the tissue thicknesses of the control arm, respectively. Repeat ultrasound examinations after 48 to 96 hours showed considerable resolution of muscle swelling, compared with subcutaneous tissue swelling. There was no significant difference in the extent of swelling detected between children who received diphtheria-tetanus-acellular pertussis vaccine and those who received reduced-antigen content diphtheria-tetanus-acellular pertussis vaccine.

CONCLUSION. Extensive limb swelling reactions after diphtheria-tetanus-acellular pertussis or reduced-antigen content booster immunizations involved swelling of subcutaneous and muscle tissues with swelling and duration more marked in subcutaneous tissue.

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Key Words
local reaction, adverse events, diphtheria, pertussis, tetanus, ultrasound

Abbreviations
DTaP—diphtheria-tetanus-acellular pertussis
Tdap—reduced-antigen content diphtheria-tetanus-acellular pertussis
DTwP—diphtheria-tetanus-whole-cell pertussis
ELS—extensive limb swelling
ISR—injection site reaction
CI—confidence interval

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IMMUNIZATION IS A key primary prevention activity that has assisted significantly in the reduction of rates of childhood morbidity and premature death. Important components of a responsible immunization program are investigation and prevention of adverse vaccine reactions.[1] In Australia, commencing in 1997, the National Health and Medical Research Council recommended a 5-dose course of diphtheria-tetanus-acellular pertussis (DTaP) vaccine as part of the childhood immunization schedule.[2] This schedule included a primary 3-dose course of DTaP vaccine at 2, 4, and 6 months of age, a fourth dose at 18 months, and a fifth dose between 4 and 6 years of age.

Before 1997, diphtheria-tetanus-whole-cell pertussis (DTwP) vaccine was the recommended vaccine for all 5 doses. DTaP vaccine became the vaccine of choice because of an improved reactogenicity profile, compared with DTwP vaccine.[2] Through passive surveillance of adverse events after immunization in South Australia, an increase in the rate of injection site reactions (ISRs) after the fourth dose of DTaP vaccine and an increased relative risk of an ISR after DTaP primary immunization were observed, in comparison with DTwP immunization.[3] Similarly, in other countries, increases in the rates and severity of ISRs were observed with successive doses of DTaP vaccine.[4–13] Previous studies indicated that the incidence ranged from 2% to 24% depending on the definition of extensive swelling reaction used.[3–13] In contrast, rates of systemic reactions seemed to remain constant or even to decrease with later DTaP vaccine doses.[14] The pathogenesis of ISRs is complex, probably multifactorial, and not fully understood.[7,8,15]

Of greater concern is the increased incidence of extensive limb swelling (ELS) reported after booster doses of DTaP vaccines.[4,9,11–13] A variety of definitions of ELS reactions exist, including use of an arbitrarily defined cutoff measurement of superficial redness and/or swelling at the site of injection. In the *Australian Immunisation Handbook*, an ELS reaction is defined as swelling and/or redness over a substantial area, involving at least one half of the circumference of the limb and involving an adjacent joint above or below the injection site, commencing within 48 hours after immunization and resolving completely without sequelae.[14] This type of reaction is not unique to DTaP vaccines and has been observed after administration of other vaccines, such as DTwP and hepatitis B vaccines.[7–9]

The reduced-antigen content diphtheria-tetanus-acellular pertussis (Tdap) vaccine was shown to result in less-extensive ISRs when used as booster immunizations for 4- to 6-year-old children who were primed with DTwP.[16] A Tdap vaccine (Boostrix [GlaxoSmithKline Biologicals, Rixensart, Belgium], containing >2 IU of diphtheria toxoid, >20 IU of tetanus toxoid, 8 μg of pertussis toxoid, 8 μg of filamentous hemagglutinin, and 2.5 μg of pertactin) is licensed currently in many countries for use as a booster vaccine for adults and children from 10 years of age. It is not known whether there are likely to be fewer or less-severe reactions with a Tdap vaccine, compared with DTaP vaccine (Infanrix [GlaxoSmithKline Biologicals], containing 30 IU of diphtheria toxoid, 40 IU of tetanus toxoid, 25 μg of pertussis toxoid, 25 μg of filamentous hemagglutinin, and 8 μg of pertactin), when administered to 4- to 6-year-old children who were primed with DTaP vaccine and who experienced an ISR at 18 months of age.

The aim of this study was to use clinical and ultrasound examinations to determine the site, extent, and resolution of tissue involvement in the injected limb for children who developed an ELS response to DTaP or Tdap vaccination at 4 to 6 years of age. Ultrasonography was used previously to measure the thickness of subcutaneous and muscle layers, to determine appropriate needle length for intramuscular injections,[17–19] but has not been used for formal assessment of ISRs after DTaP or Tdap immunization.

METHODS

Subjects

As part of a larger, double-blind, prospective study conducted at the Women's and Children's Hospital with children who had experienced a previous ISR after DTaP vaccine administration at 18 months of age, 4- to 6-year-old children ($n = 25$) were assigned randomly to receive either DTaP vaccine ($n = 13$) or Tdap vaccine ($n = 12$). A previous ISR was defined as a history of swelling or redness centered at the site of injection, with the addition of one of the following: swelling to the nearest joint (with or without redness), swelling extending from joint to joint (with or without redness), swelling of >3-day duration (with or without redness), and/or requirement for hospitalization and/or medical attention including review by a medical practitioner. Children were enrolled from the Special Immunisation Service at the Women's and Children's Hospital and through the state scheme for surveillance of adverse events after immunization at the South Australian Immunisation Coordination Unit, Department of Health (Adelaide, South Australia). A letter was mailed to all families with a child who was reported as experiencing a large ISR after the 18-month DTaP vaccination. In South Australia, in addition to medical officer reporting, parental reporting of any adverse reaction to a vaccine is encouraged. Participation in the ultrasound study depended on the availability of the ultrasonographer at the time the ELS reaction was assessed clinically and provision of informed consent from the subject's parent/legal guardian.

Healthy children who had experienced an ISR at 18 months of age and who had received 4 doses of DTaP vaccine previously (at 2, 4, 6, and 18 months of age) were enrolled in the study. Subjects were excluded if

they had received any vaccine or nonregistered drug within 30 days before study commencement, had an immunodeficiency condition, had evidence of previous or intercurrent diphtheria, tetanus, or pertussis disease or vaccination against any of these diseases since booster immunization in the second year of life, or had a history of an allergic disease that was likely to be exacerbated by any component of the study vaccine. Subjects were assigned randomly to receive either DTaP or Tdap vaccine, which was administered intramuscularly by the investigator team into the deltoid muscle of the left arm, with a 23-gauge, 25-mm-long needle, at an angle of 60°. The needle was inserted in the middle of the deltoid muscle, halfway between the acromion and the insertion of the deltoid muscle.

Reactogenicity

Solicited local and general symptoms were collected during a 15-day follow-up period (the day of immunization and 14 consecutive days). Solicited local signs of redness, swelling and increased mid-upper arm circumference, and pain localized to the injection site and solicited general symptoms of fever, irritability, drowsiness, and loss of appetite after immunization were recorded daily by parents on diary cards. The maximal swelling, redness, and induration of the affected upper arm were measured in millimeters by parents/caregivers, who were provided with a standardized, clear, flexible ruler. The circumference of the arm was measured by parents at a previously defined midhumeral point. The midhumeral point was defined as the midpoint between the acromion process and the lateral epicondyle of the humerus. This point was determined at the first appointment and was marked with a semipermanent tattoo. Parents were asked to complete a 4-point graded scale for pain (grade 0 = no pain, grade 1 = minor reaction to touch, grade 2 = protests on touch, and grade 3 = spontaneously painful) and for functional impairment (grade 0 = no impairment, grade 1 = easily tolerated, normal activity, grade 2 = discomfort, interferes with normal activity, and grade 3 = prevents normal activity) and to record the maximal grade daily.

Parents were instructed to inspect the injection site at the same time each day and to measure the size of any redness and/or swelling at the injection site. Parents were asked to notify study staff members if the child developed either redness or swelling of >50 mm (largest diameter) at the injection site, a >30-mm increase in injected limb circumference at the midhumeral point (compared with baseline), or any functional impairment of the arm. These screening criteria were used to ensure that all cases of suspected ELS were assessed by a medical officer. The child was examined by a study medical officer within 24 hours after notification by the parent/guardian. Ultrasound examinations were performed after clinical examination of the ELS reaction 24 to 48 hours after immunization and were repeated 48 to 96 hours after the first ultrasound assessment. The ultrasound assessments were blinded with respect to use of Tdap or DTaP vaccine.

Ultrasound Examinations

A linear-array transducer was used to assess the extent of swelling in the upper arm. The examination involved 6 transverse views at 2-cm intervals and 3 longitudinal views (in 9 segments). Aquasonic contact gel (Parker Laboratories, Fairfield, NJ) was applied to the skin, and a marker was used to define the areas for examination. Subcutaneous tissue thickness and muscle thickness were measured in both the affected arm and the nonaffected arm, and the absolute values were compared. Ultrasound examination of the joint was performed for children with clinically apparent swelling extending to a joint. A comparison between the clinically determined estimate of swelling extent and a measurement of the extent of edema of the tissues was undertaken during the ultrasound examination. A repeat ultrasound examination was performed 48 to 96 hours later, and a comparison between the initial and follow-up ultrasound examination findings was made to delineate changes to tissues during resolution of the local reaction.

Statistical Analyses

Fisher's exact test was used for comparisons of the incidence and severity of reactivity. The increase in thickness of muscle and subcutaneous tissue in the vaccinated arm, compared with the control arm, was reported as a proportional (percentage) increase in thickness, with the control arm being 100%. The differences in means for subcutaneous and muscle swelling in the 2 groups were compared by using an unpaired t test, assuming equal variance (Stata software, release 8.2; Stata Corp, College Station, TX). The appropriate t test was determined by using the Levene statistic to assess variance in the means between the 2 groups. The difference in thickness of muscle and subcutaneous tissue 48 to 96 hours after the initial ultrasound examination was also determined.

This study was conducted in the Department of Paediatrics and the Department of Medical Imaging at the Women's and Children's Hospital in Adelaide, South Australia. Both the primary study and the ultrasound study were approved by the Women's and Children's Hospital Research Ethics Committee, and informed consent was obtained before any study procedures were performed. The study was conducted according to the Declaration of Helsinki and good clinical practice.

RESULTS

Study Population

The study was conducted between March 2003 and June 2004. Twelve children (8 boys and 4 girls) who devel-

oped an ISR after either DTaP or Tdap vaccination were enrolled in the ultrasound study. All children enrolled in the study had redness and swelling of >50 mm at the site of injection and were considered to have an ELS reaction. Figures 1 and 2 show the clinical appearance of a typical ELS reaction. With unblinding, it was determined that 8 of the 12 subjects enrolled in the ultrasound study had received DTaP vaccine and 4 subjects had received Tdap vaccine. All children were of white origin, with a mean age of 4.4 years (Tdap: 4.7 years; DTaP: 4.3 years; range: 4–5.75 years).

Parental Reporting of General Symptoms

All children reported grade 1 to 3 pain, with only 2 subjects, 1 from each group, reporting grade 3 pain. Only 1 subject (DTaP group) reported grade 3 functional impairment, with 5 subjects reporting grade 1 to 2 functional impairment in the DTaP group ($n = 8$) and 2 subjects reporting grade 1 functional impairment in the Tdap group ($n = 4$). With Fisher's exact test, there was no difference in the degree of functional impairment between the 2 vaccine groups ($P = .999$).

Three subjects in each group reported grade 1 fever. Equal proportions of children in each group reported irritability, drowsiness, and loss of appetite. Seven subjects required paracetamol (acetaminophen) to relieve symptoms associated with the immunization, ranging from 1 to 6 doses in total. Children who required >3 doses of paracetamol were male and had received DTaP vaccine. The child who reported grade 3 pain also reported grade 2 functional impairment and required 5 doses of paracetamol for adequate pain relief.

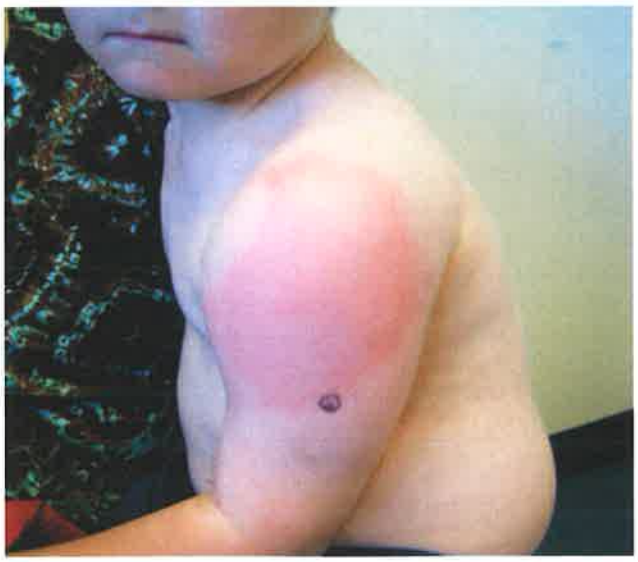

FIGURE 1
ELS reaction of the left deltoid and shoulder region. The semipermanent mark on the child is the midhumeral point (midway between the acromion and the distal part of the lateral epicondyle of the humerus).

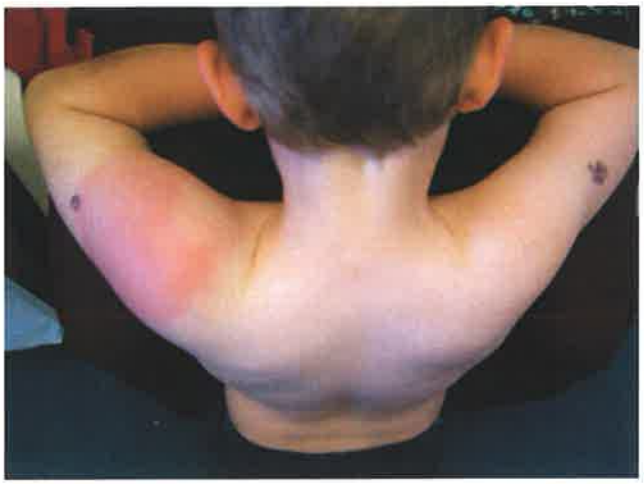

FIGURE 2
ELS reaction of the left deltoid and shoulder region.

Parental Reporting of Local Symptoms

The extent of clinically measured redness and swelling in the injected limb for children who received DTaP and Tdap vaccines is recorded in Tables 1 and 2. Six of the 12 children developed redness at the immunization site within 24 hours after immunization. Five of these children were in the DTaP group. The onset of swelling occurred as early as 6 hours after immunization, with the total duration of swelling being 2 to 8 days. The onset of swelling occurred on the day of immunization for 3 subjects, on day 1 for 6 subjects, and on day 2 for 2 subjects. Four subjects developed their maximal swelling by day 1 and the remaining 8 subjects by day 2. All subjects developed redness and/or swelling of >50 mm within 48 hours after immunization. All recovered without sequelae. There were no serious adverse events reported for any children enrolled in the ultrasound study; specifically, no hospitalization was required for management of ELS reactions.

Clinical Assessment of Swelling by Medical Officer

Nine subjects (DTaP, $n = 7$; Tdap, $n = 2$) had swelling of the deltoid region extending to the shoulder, 2 subjects (DTaP, $n = 1$; Tdap, $n = 1$) had swelling localized to the injection site, and 1 subject (Tdap group) had diffuse swelling of the upper arm. One of the 4 subjects in the Tdap group had swelling extending from beyond the shoulder joint to the cubital fossa but not encompassing the elbow joint.

Ultrasound Examinations 24 to 48 Hours After Immunization

Ultrasound examinations showed a diffuse, echogenic, "snowstorm" appearance, consistent with diffuse edema of the tissues (Figs 3 and 4). All children showed evidence of edema in both subcutaneous and muscle tissues, extending to the humeral cortex. Subcutaneous

TABLE 1 Clinical and Ultrasound Measurements of ELS Reactions for Individual Subjects

Subject No.	Gender	Vaccine	Redness Diameter, mm[a]	Swelling[a]		Ultrasound Assessment of Increase in Tissue Swelling, mm[b]	
				Diameter, mm	Duration, d	Subcutaneous Tissue	Muscle Tissue
1	Male	DTaP	350	350	5	7.3	6.5
2	Female	Tdap	175	175	8	7.0	4.2
3	Male	DTaP	120	110	4	8.9	1.6
4	Male	DTaP	160	70	3	7.8	6.3
5	Male	Tdap	60	60	3	4.9	1.1
6	Female	DTaP	80	55	2	7.1	1.4
7	Male	DTaP	140	95	3	4.6	10.0
8	Male	Tdap	202	245	8	5.0	8.0
9	Male	DTaP	170	160	8	4.4	2.0
10	Female	DTaP	94	106	5	4.1	6.3
11	Male	DTaP	140	180	4	11.8	2.0
12	Female	Tdap	64	90	7	5.1	1.6

[a] Maximal measurement recorded by parents on diary cards on days 0 to 14 after vaccination.
[b] Subcutaneous and muscle thickness measurement = vaccinated arm − control arm.

TABLE 2 Measurement of ELS Parameters Recorded on Diary Cards by Parents for Children ($n = 12$) Immunized With DTaP or Tdap, During the 15-Day Follow-Up Period

	DTaP ($n = 8$)	Tdap ($n = 4$)	Difference, P	Total
Redness diameter, mm				
Mean (95% CI)	169.3 (95.4–243.0)	103.0 (−2.6 to 208.0)	.18[a]	147.2 (93.1–201.0)
Range[b]	80.0–350.0	60.0–202.0		60.0–350.0
Swelling diameter, mm				
Mean (95% CI)	150.8 (64.7–236.8)	147.0 (18.8–275.2)	.95	149.5 (90.9–208.1)
Range[b]	55.0–350.0	60.0–245.0		55.0–350.0
Increase in limb circumference, mm[c]				
Mean (95% CI)	30.9 (15.0–46.7)	24.5 (−3.6 to 29.3)	.61	27.9 (16.5–39.4)
Range	7.0–64.0	8.0–56.0		7.0–64.0
Proportional increase in limb circumference, %[d]				
Mean (95% CI)	16.2 (8.7–23.7)	12.9 (−3.6 to 29.3)	.58	15.1 (9.3–20.9)
Range	4.0–31.1	4.4–27.7		4.0–31.1

[a] Two-sample, unpaired t test with unequal variances.
[b] Range of individual measurements of maximal redness and swelling during the observation period.
[c] Increase in arm circumference = maximal arm circumference − baseline circumference.
[d] Proportional increase = increase in arm circumference/baseline circumference, with baseline circumference being the circumference of the arm immediately before vaccine administration.

and muscle tissues expanded to a maximum of 281% and 111% of the tissue thickness of the control arm, respectively. No fluid was detected in the shoulder joint for children who clinically exhibited swelling that extended over the shoulder joint. The changes in subcutaneous and muscle tissue are detailed in Tables 1 and 3 and Fig 5. All children developed swelling of subcutaneous and muscle tissues. For subcutaneous tissue swelling, 42% of subjects had swelling of ≤5 mm, 50% had swelling of 5.1 to 10 mm, and 8% had swelling of 10.1 to 15 mm. For muscle tissue swelling, 58% of subjects had swelling of ≤5 mm and 42% had swelling of 5.1 to 10 mm.

The mean percentage increase in swelling of subcutaneous tissue for children who received DTaP vaccine ($n = 8$) was 136.0% (95% confidence interval [CI]: 73.1%–198.0%), compared with 124.3% (95% CI: 66.5%–182.0%) for children who received Tdap vaccine ($n = 4$). The unpaired Student's t test was used to test the hypothesis that the mean increases in subcutaneous and muscle thickness were equivalent in the 2 groups (subcutaneous swelling, $P = .78$; muscle swelling, $P = .945$). The variances in the means were equivalent in the 2 groups for subcutaneous (Levene statistic, $P = .485$) and muscle (Levene statistic, $P = .434$) swelling.

When clinical measurement of swelling by the medical officer, with the use of superficial landmarks, was compared with ultrasound assessment of the extent of deeper tissue swelling, the measurement obtained during the ultrasound examination exceeded the clinical assessment of swelling for 10 of the 12 subjects (Fig 6).

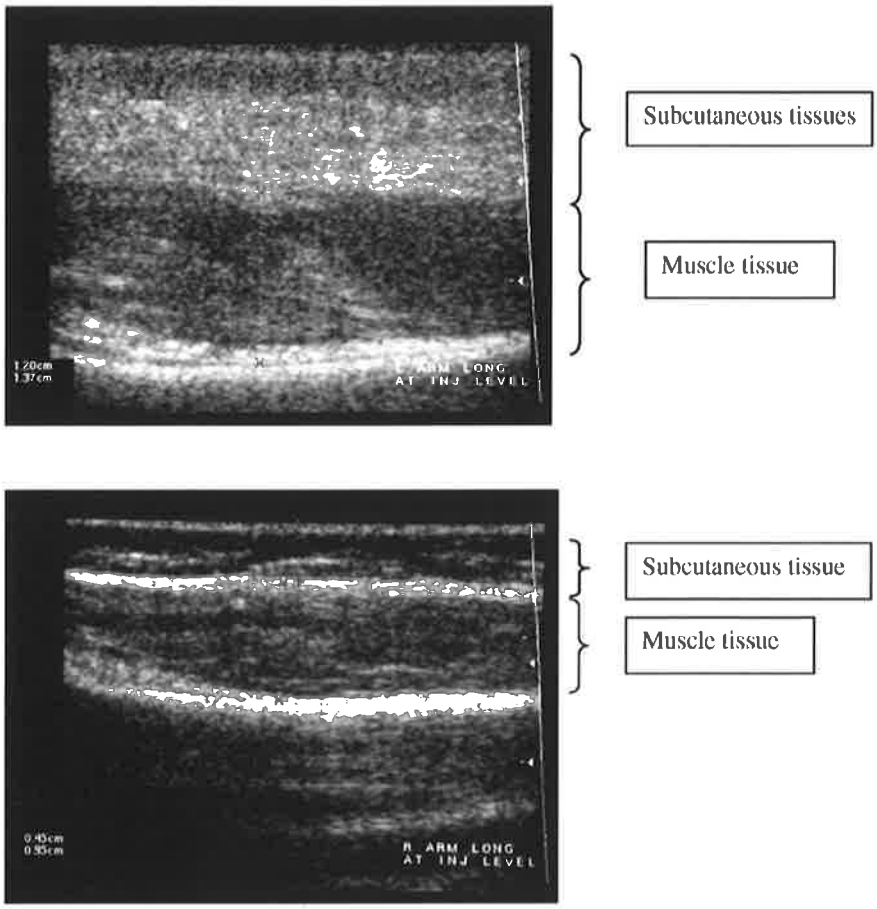

FIGURE 3
Ultrasound examination of a child with ELS of the left arm. The right arm (no injection) is shown for comparison.

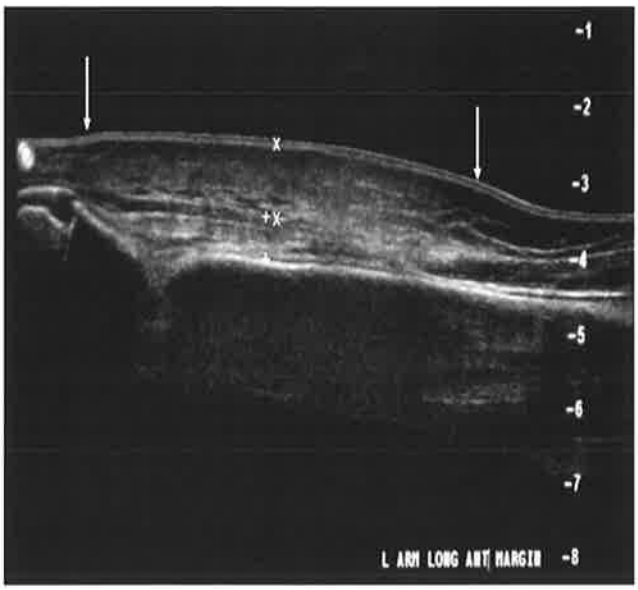

FIGURE 4
Lateral view of ELS reaction in the left upper arm. Arrows indicate the extent of subcutaneous tissue and muscle swelling in the upper arm. x indicates swelling in subcutaneous tissue; +, swelling in muscle tissue.

Repeat Ultrasound Examinations 48 to 96 Hours After Initial Examinations

Eleven of the 12 subjects enrolled had a repeat ultrasound examination performed (1 subject failed to present for the second ultrasound assessment). Swelling in muscle tissue resolved more rapidly than the subcutaneous tissue swelling, as shown in Fig 5. Fifty percent ($n = 6$) of subjects had resolution of muscle swelling, compared with only 8.3% ($n = 1$) with resolution of subcutaneous swelling.

DISCUSSION

An ELS reaction after booster doses of the acellular pertussis combination vaccines can seem alarming to parents and vaccine providers. If this reaction is not recognized, there may be an incorrect diagnosis of infective cellulitis, resulting in inappropriate treatment with antibiotics. This descriptive study demonstrated that the ELS reaction was attributable to marked edema in both the subcutaneous and muscle tissue spaces, with fluid accumulation being greater in the subcutaneous tissue space. It is interesting that there was significant subcutaneous edema, given that the vaccines were administered through the intramuscular route. The swelling of muscle tissue was generally not as extensive and seemed to resolve more rapidly than the swelling in subcutaneous tissue; this might be related to the better blood supply to muscle, compared with subcutaneous tissue. Despite the extensive swelling, there was no evidence of associated joint effusion.

TABLE 3 Comparison of Subcutaneous Tissue and Muscle Swelling, Measured With Ultrasonography, at 24 to 48 Hours for Children Who Received DTaP or Tdap

	DTaP		Tdap		Difference in Mean Tissue Thickness for DTaP and Tdap[a]		All Subjects	
	Increase, mm	Proportional Increase, %	Increase, mm	Proportional Increase, %	Increase, *P*	Proportional Increase, *P*	Increase, mm	Proportional Increase, %
Increase in subcutaneous tissue thickness[b]								
Mean (95% CI)	7.0 (4.81–9.22)	136.0 (73.1–198.0)	5.5 (3.9–7.1)	124.3 (66.5–181.0)	.30	.78	6.5 (5.1–8.0)	132.1 (91.9–172.3)
Range[c]	4.1–11.8	62.0–281.0	4.9–7.0	104.0–140.0			4.1–11.8	62.0–281.0
Increase in muscle thickness[d]								
Mean (95% CI)	4.3 (1.4–7.2)	43.6 (12.8–74.0)	3.7 (−1.3 to 8.7)	45.3 (−16.1 to 106.0)	.79	.94	4.1 (2.1–6.1)	44.2 (21.5–66.8)
Range	0.2–10.0	2.0–111.0	1.1–8.0	15.0–100.0			0.2–10.0	2.0–111.0

[a] Diffference in increase in tissue thickness = DTaP tissue thickness/Tdap tissue thickness.

[b] Increase in subcutaneous tissue thickness = subcutaneous tissue thickness in vaccinated arm − subcutaneous thickness in control arm.

[c] Minimal and maximal ultrasound measurements.

[d] Increase in muscle thickness = muscle thickness in vaccinated arm − muscle thickness in control arm.

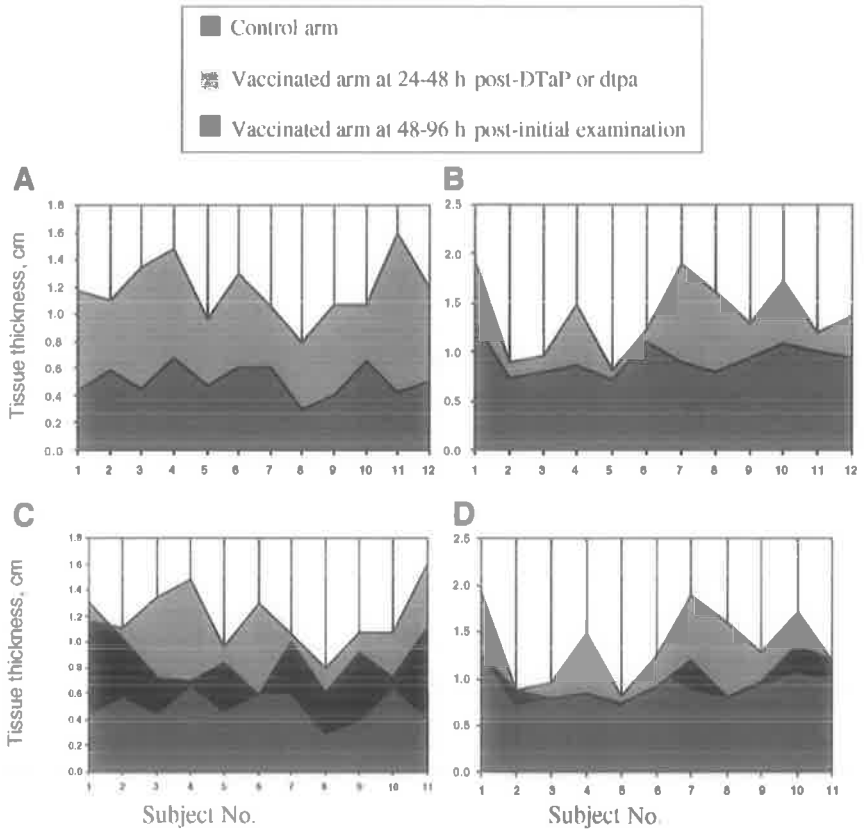

FIGURE 5
Ultrasound measurements of subcutaneous and muscle tissue after DTaP or Tdap immunization. A, Subcutaneous tissue; B, muscle tissue; C, subcutaneous tissue 48 to 96 hours after the initial examination; D, muscle tissue 48 to 96 hours after the initial examination. Subject 12 did not present for the follow-up ultrasound examination.

The mechanism of ELS has not yet been defined clearly, with no apparent risk factors being evident, other than priming with previous DTaP vaccines. The characteristics of the swelling suggest angioedema rather than inflammatory cellulitis, given the absence of systemic fever and pain and the rapid resolution of swelling. Angioedema results from altered vascular permeability and can occur through a variety of mechanisms, including uninhibited activation of the complement pathway and mast cell degranulation, with release of vasoactive peptides. Additional research is required to determine the mechanism of ELS after vaccination. The pathogenesis of ELS is likely to be multifactorial. Several DTaP vaccine components (eg, aluminum, diphtheria toxoid, and pertussis toxoid) have already been shown to be associated with increased reactogenicity.[7] High prevaccination antibody levels against ≥1 antigen present in the vaccine, IgE antibody levels, and cell-mediated

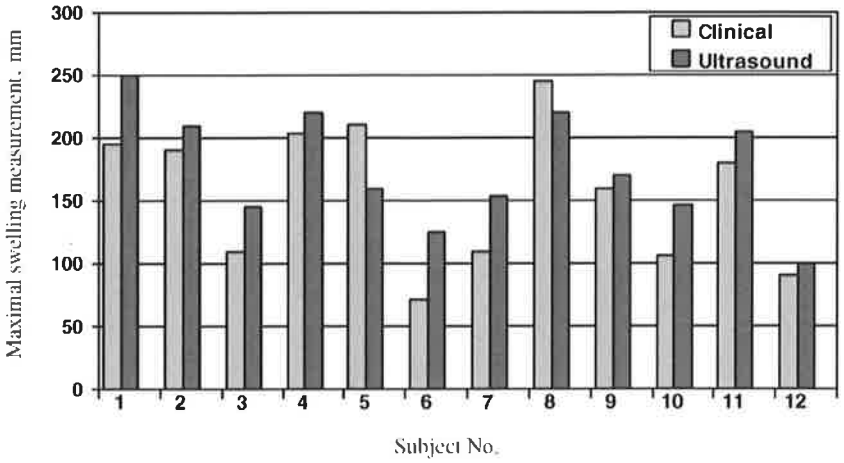

FIGURE 6
Clinical versus ultrasound measurements of ELS reactions at the initial ultrasound examination. Clinical measurements of swelling by the medical officer with the use of superficial landmarks were compared with ultrasound assessments of the extent of deeper tissue swelling.

responses are other factors that may contribute to the increase in ELS reactions.[8,15]

Children who received primary doses of DTaP vaccine seemed to be at higher risk of experiencing an ELS reaction with booster doses of either diphtheria/tetanus/pertussis or DTaP vaccine.[9,20,21] In this study, ELS reactions were seen with both DTaP and Tdap vaccine administration for the booster dose at 4 to 6 years of age.

Despite the size of ELS reactions, generally children experience only a mild degree of functional impairment or pain significant enough to require analgesia. This is supported by the outcomes of other studies that showed similar degrees of morbidity associated with large injection site reactions. In the study by Scheifele et al,[22] 19.3% of children who received diphtheria/tetanus/acellular pertussis/inactivated polio virus vaccine as a fifth dose developed redness or swelling of 50 mm that required an average of 5 days to resolve, with the largest reactions requiring up to 10 days. None of the children required medical attention. In a survey of 800 parents of children who received a fifth dose of acellular pertussis vaccine, the incidence of redness larger than an Oreo cookie was 25%.[10] None of the children required hospitalization, and few children needed to interrupt educational activities after immunization.

All subjects who fulfilled the study criteria of a previous ISR at 18 months developed an ELS reaction after the 4-year booster dose of DTaP or Tdap vaccine. It should be noted that these criteria were likely to include children with less-severe reactions than might be included in other studies. If the definition provided in the *Australian Immunisation Handbook* is used, then 9 of the 12 subjects enrolled had swelling consistent with a diagnosis of ELS (including swelling at an adjacent joint). Because of the small sample size in this study, no inference can be made about the risk of recurrence of ISRs, apart from the statement that the risk seemed high in our study population. Although the selection process had the potential to lead to selection bias, when clinical estimates of ELS reactions were compared between subjects who participated in the ultrasound study and those who did not, there was no statistically significant difference between the 2 groups. Children who received an ultrasound examination were therefore representative of all children enrolled in the prospective study of ISRs at our study center ($n = 25$).

To our knowledge, ultrasonography has not been used previously to measure the extent of ELS reactions. Standardization of adverse event definitions is essential for accurate surveillance and reporting of vaccine-associated adverse events. The Brighton Collaboration is in the process of establishing definitions for vaccine-associated adverse events. Establishing a standardized definition for ELS reactions is required[1] and will be important for both passive and active surveillance of adverse events after immunization, because a consistent definition will allow comparisons of different vaccine trials and surveillance systems.

Despite the increased local reactogenicity of booster doses, acellular pertussis combination vaccines remain the preferred vaccines for preventing pertussis, diphtheria, and tetanus for children because of the improved safety profile, compared with the more-serious systemic adverse events that occur more frequently with whole-cell pertussis vaccines. However, demonstration of an increase in ISRs after booster doses of DTaP vaccine was one of the factors that resulted in the 18-month booster dose being omitted from the Australian Standard Vaccination Schedule in 2003.[3,4] The decision was also based on the prolonged immunity now known to result from a primary course of DTaP vaccine treatment.[23] Provision of Tdap vaccine as an alternative may provide a safer alternative for children who developed an ELS reaction with previous booster doses. There are several formulations of Tdap vaccine available. Studies comparing adverse events after vaccination with a Tdap vaccine (5 flocculation units of tetanus toxoid, 2 flocculation units of diphtheria toxoid, 2.5 μg of pertussis toxoid, 5 μg of

filamentous hemagglutinin, 3 μg of pertactin, and 5 μg of fimbriae types 2 and 3) or diphtheria/tetanus vaccine in 11- to 17-year-old subjects showed a lower incidence of ISRs (swelling of ≥50 mm) in the Tdap vaccine group, compared with the diphtheria/tetanus vaccine group (2.8% and 3.6%, respectively; Aventis Pasteur, unpublished data). We were unable to demonstrate any significant benefit from Tdap vaccine use in our study, but we acknowledge that the study was descriptive in design and was not powered to detect a difference between the 2 groups.

Surveillance, acknowledgment, and transparency in relation to vaccine-associated adverse events are essential to ensure public confidence in immunization programs. Additional research and education for providers and consumers about the nature and cause of adverse reactions such as those described in this study should be a priority.

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Chapter 5: New Vaccine Schedules

Despite an established and effective immunisation program for children and adults in Australia, data on epidemiology of infectious diseases suggests that some infections are not yet optimally controlled. Pertussis and varicella are examples of funded vaccine programs that have been unsuccessful in eliminating the infectious agent. Pertussis epidemics continue to emerge every 3-4 years particularly affecting adults but also causing deaths in infants too young to be vaccinated. Varicella breakthrough disease suggests a single dose of vaccine is not sufficient to eliminate this infection. By monitoring disease incidence and populations affected, immunisation programs can be modified to improve disease control.

Pertussis Immunisation at birth

In Australia during 2008 – 2009, we have experienced a severe pertussis epidemic associated with infant deaths, confirming that protection from the routine 2, 4, 6 month immunisation schedule for pertussis is suboptimal. In order to provide protection for the youngest most vulnerable infants alternative vaccination schedules are being considered including pertussis vaccination at birth, maternal immunisation (during pregnancy), immunising parents prior to conception or immediately after delivery, "cocooning" and introducing 10 yearly boosters for adults.

High morbidity and mortality from pertussis has been recognised for the past century with early trials of maternal and neonatal vaccination with whole cell vaccine preparations being conducted in the 1940s. However, following the suggestion that pertussis immunisation in the neonatal period could induce immune tolerance the emphasis shifted to commencing whole cell vaccines later in the first year of life. Three small studies examining administration of pertussis-containing vaccines in the first week of life have recently been published with conflicting results. Two studies of monovalent pertussis (Pa) vaccine given at birth suggested that earlier antibody responses could be achieved without induction of immune tolerance, while one using DTPa at birth suggested the possibility of developing immune tolerance.

Our research unit has been involved in studies of neonatal pertussis vaccination to determine whether it is safe and immunogenic to provide earlier protection for those at highest risk of death.

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16. White OJ, Rowe J, Richmond P, **Marshall H**, McIntyre P, Wood N, Holt PG. Th2-polarisation of cellular immune memory to neonatal pertussis vaccination. Vaccine (Accepted for publication 5 August 2009)

These two papers describe the results of a study to assess a novel pertussis vaccination schedule in early infancy with wide potential application to vaccination programs worldwide.

Paper 15 describes the results of the first study conducted to assess the immunological and clinical outcomes of two doses of an acellular pertussis vaccine prior to 8 weeks of age (birth and 4 weeks old).

This was the first study to assess the immunogenicity and reactogenicity of two doses of Pa vaccine (birth and 1 month) given before 2 months of age. The study was also unique in that all infants received HBV vaccine at birth, thus allowing direct comparison of the potential influence of birth Pa vaccine on concomitant hepatitis B responses.

This study showed statistically significant higher pertussis (anti-pertussis toxin (PT), anti-pertactin (PRN) and anti-filamentous haemagglutinin (FHA)) IgG antibodies at two months of age in infants who received two doses of Pa prior to two months of age, compared to those receiving Pa at birth only or no previous Pa vaccine. The levels of anti PT and anti PRN IgG achieved after doses at birth, one and two months of age (i.e. 3 separate doses) were similar to those seen with 3 doses at 0, 2, 4 and 2, 4, 6 months. This suggests that clinically significant protection against severe pertussis could be achieved 4 months earlier than under current vaccination schedules. Our study also suggested that a first dose at birth primes the immune system, with a significant increase in antibody after the second dose, whether given at one or two months of age. Importantly, four doses of a Pa-containing vaccine within 4 months did not result in local or systemic adverse events, albeit the small sample size. Pa vaccine given at birth was well tolerated with no increase in reactogenicity identified at birth or with subsequent vaccine doses (2, 4, 6 months old) compared to infants receiving the routine vaccine schedule, similar to other studies of pertussis-containing vaccines at birth. Immune tolerance was not seen in our study.

Previous studies have shown that high levels of maternal antibodies to pertussis can interfere with subsequent infant responses. In our study, the impact of maternal pertussis antibody, particularly higher levels, on responses to Pa vaccines at birth or subsequently remains uncertain as our sample size was small. Within these power limitations, we did not see any trend to suggest interference from maternal antibody. In our study Hib (anti-PRP) IgG levels at 7 months of age were non-significantly lower in the birth Pa and one month old Pa group. Reduced anti-PRP IgG responses have been associated with DTPa-Hib combination vaccines as discussed in Chapter 1, but this has only emerged as a clinical problem in the UK prior to introduction of a Hib booster and so may not be clinically relevant if a booster is routinely given. Although reduced Hepatitis B (anti HBs) antibody GMC was seen in infants receiving Pa at birth, all participants achieved anti-HBs levels above the protective level (anti-Hbs >10 mIU/ml) at 8 months of age. There is unlikely to be any clinical relevance to the reduced anti-PRP and anti-HBs but this will be examined further in a larger NHMRC funded study to be conducted this year (as described below).

The possibilities of later reductions in antibody response, and/or interference with responses to concomitantly administered antigens, necessitates larger studies, and raise important additional questions. These include the timing of the second dose of pertussis-containing vaccine. A second dose at 6 weeks of age would be feasible and practical, as current combination vaccines including Pa are licensed from this age and 6 weeks is consistent with the current WHO schedule. Our future study with a larger sample size will address the influence of high maternal antibodies on infant pertussis responses, as might be achieved following adult or adolescent dTpa booster doses.

Paper 16 describes cell mediated immunity responses in children receiving pertussis immunisation at birth (previously described in Paper 15). The cell mediated immune responses were measured by Professor Pat Holt, Telethon Institute of Child Health, Perth, Western Australia. Previous studies have shown that acellular pertussis-containing DTPa vaccine induces cellular immune memory, which is strongly polarised towards the Th2 phenotype in infants and preschoolers. This has the theoretical potential to negatively impact on immune responses to co-administered Th1 inducing vaccine. This may result in increased susceptibility to infection due to a suboptimal immune response. In addition, Th2 skewed immunological memory to DTPa antigens induced by infant vaccination has been shown to increase the risk for severe local reactions to subsequent booster pertussis vaccinations.

In this pilot study, we contrasted pertussis specific immune responses in infants receiving DTPa vaccine as per the standard 2-4-6 month protocol, or with additional doses of Pa vaccine at birth or at birth and 1 month of age. We assessed immunological outcomes by measuring IgG titres to three major pertussis antigens following completion of the primary immunisation course, and by measuring *in vitro* Th-memory cell cytokine responses to pertussis antigens in PBMC. Enhanced antibody titres were evident in infants who had received 2 doses of pertussis vaccine by 2 months of age, compared to 3 months, which is likely to provide protection when the infant is at most risk of severe disease or death. Further studies are required to determine whether these increased pertussis-specific IgG titres translate into reduced susceptibility to pertussis infection.

In a larger NHMRC funded study conducted by our research unit, cell mediated immune responses will be profiled in more detail, because cell mediated immunity is potentially an important component of protection and because birth Pa vaccine may result in Th2 bias, with altered responses following subsequent natural pertussis exposure or increased atopy as potential negative effects.

All study participants are currently being followed to 4 years of age in a long term follow-up study to assess adverse events and pertussis antibody responses pre and post the routine DTPa booster given at 4 years of age in Australia. The long term follow-up study was funded by a Women's and Children's Hospital Foundation Grant (2007-2008).

The study results reported in Paper 15 were presented at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, Illinois, USA, September 17 – 20, 2007 and nationally at the 9th National Public Health Association of Australia Immunisation Conference/1st Asia Pacific Vaccine Preventable Diseases Conference in Cairns, Queensland, Australia, August 19 – 20, 2004 and the Royal Australasian College of Physicians' Annual Scientific Meeting in Adelaide, South Australia, May 11 – 15, 2008.

The results reported in Paper 16 were presented at the 7th Louis Pasteur Conference on Infectious Diseases in Paris, France, November 11 – 13, 2008 and nationally at the Australasian Society of Immunology, Sydney, Australia, December 2 – 6, 2007 and the Australasian Vaccines and

Immunotherapeutics Development (AVID) Conference in Sydney, New South Wales, Australia, May 14 – 16, 2008.

17. Roberton D, Marshall H, Dinan L, Boros C, Gold M. Developmental immunology and vaccines. Expert Review of Vaccines 2004;3(4):343-347.

This paper provides a review of the literature on the optimal immunisation of preterm infants who are at increased risk of severe disease from vaccine preventable infections, compared to term infants.

Prematurity was noted to be a risk factor for the development of Hib disease during surveillance of Hib vaccine failures following the introduction of Hib immunisation in the UK. Since then, a comparatively small number of studies have assessed the safety, immunogenicity, efficacy and duration of immune responses in preterm infants compared to term infants for many of the routinely recommended childhood immunisations. In some of these studies, preterm infants have been shown to demonstrate a variable immune response to protein based antigens. However data on the newer conjugate vaccines are limited. Data from studies of the immune responses of premature infants to routine immunisation are limited both by sample size and the relatively small number of studies that have been performed in preterm infants.

Recent evidence suggests that preterm infants have significant impairment in IgG antibody responses to a number of routine immunisation antigens. Importantly, IgG antibody responses to Hib, pertussis and hepatitis B vaccines are reduced in this at risk population. The evidence suggests that these reduced antibody responses persist throughout childhood. Antibody avidity has been shown to be reduced in preterm infants, although it appears for some antigens that avidity levels approach those seen in term infants by later childhood. Some smaller preterm babies do not respond as well as term babies to Hib and hepatitis B vaccines.

The current recommendations in Australia and internationally are to immunise preterm infants at their appropriate chronological age using the routine schedule with additional booster doses to ensure long term protection.

18. Roberton D, **Marshall H**, Nolan T, Sokal E, Diez-Domingo J, Flodmark C-E, Rombo L, Lewald G, de la Flor J, Casanovas J, Verdaguer J, Mares J, Van Esso D, Dieussaert I, Stoffel M. Reactogenicity and immunogenicity profile of a two-dose combined Hepatitis A and B vaccine in 1-11 year old children. Vaccine. 2005;23:5099-5105.

Protection against Hepatitis A and Hepatitis B infection has been simplified with the licensing of a combination HepAHepB vaccine. The ability to simplify it further and provide a two dose as opposed to three dose schedule was investigated in the study described in Paper 18. A two-dose schedule for the combined vaccine instead of a three dose schedule offers benefits in terms of compliance and patient acceptability.

The primary objective of this study was to show non-inferiority with regards to reactogenicity of the combined two-dose HepAHepB vaccine with double antigen content at 0 and 6 months with the established three-dose combined HepAHepB vaccine administered at 0, 1, and 6 months. The results of this study demonstrated that both two-dose combined HepAHepB vaccine with double antigen content and established three-dose schedule were well tolerated and highly immunogenic in children aged 1 to 11 years. A good tolerability profile was documented in both age groups with both vaccines. Both vaccines and schedules provided at least 98% seroprotection against hepatitis B and 100% seroconversion against hepatitis A, one month after the end of the vaccination course (month 7).

Results from these studies indicated that this two-dose schedule could be considered an alternative for immunisation of children and adolescents who are not at immediate risk of hepatitis B infection. It is particularly justified for children and adolescents in the context of school-based immunisation programs. The two-dose schedule is likely to be cost-effective, ensuring higher coverage rates as a result of fewer injections and the avoidance of missed vaccination opportunities, a two-dose regimen offers savings in syringes, vaccine storage and cold chain, transportation, medical visits, logistics and administration costs. Considering the reduction in health care budgets, a two-dose regimen provides a less costly alternative.

I presented the results of this study at the 9th National Public Health Association of Australia (PHAA) Immunisation Conference in Melbourne, Victoria, Australia, August 19 – 20, 2004 and at the Advanced Vaccinology Course, Pasteur Merieux Institute, Anecy, France in May 10 – 21, 2004.

Long term follow-up of these children has now been completed to five years with a manuscript of the results soon to be submitted to the journal "Vaccine". I will present the results at a national infectious diseases meeting (Australasian Society of Infectious Diseases) in May in 2010.

Acellular Pertussis Vaccine at Birth and One Month Induces Antibody Responses By Two Months of Age

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Background: Infants less than 3 months of age are at highest risk of hospitalization and death from pertussis. Several studies have examined antibody responses to pertussis vaccines at birth but no previous study has evaluated 2 doses of monovalent acellular pertussis vaccine (aPV) before 2 months of age.

Methods: Seventy-six newborns were randomized at birth to 3 groups–aPV at birth and 1 month, aPV at birth, and control. All infants received hepatitis B vaccine (HBV) at birth followed at 2, 4, and 6 months by a combination vaccine including aPV, diphtheria, tetanus, *Haemophilus influenzae* type b (Hib), hepatitis B, polio antigens and 7 valent conjugate pneumococcal vaccine. IgG antibody responses to pertussis toxoid (PT), filamentous hemagglutinin (FHA), and pertactin (PRN) were measured in maternal serum and in infants at 2, 4, 6, and 8 months of age. Antibody responses to hepatitis B, diphtheria, tetanus, and Hib were measured at 8 months only. A parental diary and active telephone follow-up occurred for 7 days after each vaccination.

Results: The aPV birth dose was well tolerated. By 2 months of age, 22 of 25 (88%) of 2 dose recipients had detectable IgG antibody to PT (IgG PT) compared with 9 of 21 (43%) who received a birth dose only and 3 of 20 (15%) of controls. Infants in the 2 dose group had a geometric mean concentration (GMC) of IgG PT of 16 ELISA units per mL (EU/mL), 95% CI: 11 to 25, significantly higher than birth dose only (5 EU/mL, 95% CI: 3–8) and controls (3 EU/mL, 95% CI: 2–5). At 8 months of age, following 5, 4, and 3 doses of aP-containing vaccine, respectively, IgG PT had plateaued but IgG to FHA and PRN increased with successive doses. There was a trend to lower antibody responses for hepatitis B and Hib with higher numbers of Pa doses.

Conclusion: These data suggest that aPV at birth and 1 month induces significantly higher IgG antibody against pertussis antigens by 2 months of age without reducing subsequent pertussis antibody responses. Larger and more detailed studies of aPV from birth are needed to evaluate other antibody responses and the potential of this approach to reduce death and morbidity from *Bordetella pertussis* infection in the first 3 months of life.

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Pertussis is a significant cause of mortality in early infancy worldwide. Nearly 300,000 deaths occur each year, most in developing countries, but deaths are probably underestimated in both rich and poor countries.[1,2] Death and hospitalization from pertussis occur predominantly in infants too young to receive more than 1 dose under current schedules, with over 80% of 145 reported deaths in the United States, between 2000 and 2006, occurring under 3 months of age.[3] Two doses of a pertussis-containing vaccine provide significant protection against severe disease, and even 1 dose may provide some protection against death.[4,5] The earliest age at which the first pertussis vaccine dose is currently recommended is 6 weeks under the Expanded Programme of Immunization (EPI) schedule of the World Health Organization (WHO) and can be given from 6 weeks in Europe, North America, and Australia. The second dose is given at 10 weeks under the EPI, 12 weeks in some European countries and 16 weeks elsewhere.[6] This means that, even if optimally delivered, current pertussis immunization schedules cannot provide direct protection to infants less than 8 weeks of age. When delays in immunization are taken into account, protection is often delayed even more.[7,8]

High infant morbidity and mortality from pertussis in infants was recognized more than 60 years ago,[3,9] leading to trials of maternal[10–13] and neonatal vaccination[14–17] with whole cell vaccine preparations. Following the suggestion that pertussis immunization in the neonatal period could induce immune tolerance,[16] the emphasis shifted to commencing whole cell vaccines later in the first year, even though the validity of these concerns was later questioned.[18] Currently, only BCG and hepatitis B vaccines are routinely administered at birth and their inclusion in the WHO's EPI schedule and many national vaccination programs is well-established as safe, feasible, and effective.[6,19]

Strategies to prevent early infant pertussis include universal adult and adolescent vaccination, "cocoon" vaccination of those in close contact with infants, maternal vaccination, and neonatal vaccination. No studies of maternal acellular pertussis vaccination have been published and the results of 3 recent small studies examining administration of acellular pertussis-containing vaccines in the first week of life are conflicting.[20–22] Two studies using different monovalent acellular pertussis vaccines at birth suggested that earlier antibody responses could be achieved,[20,22] but the study which used a combined diphtheria-tetanus-acellular pertussis (DTPa) vaccine at birth[21] showed inferior later antibody responses. We report the immunologic and clinical outcomes comparing 2 doses of a monovalent acellular pertussis vaccine (Glaxo Smith Kline, Belgium) at birth and 4 weeks of age with monovalent acellular pertussis vaccine at birth only and standard practice.

METHODS

Design

This pilot study was a randomized, nonblinded trial of administration of monovalent acellular pertussis vaccine (aPV) to newborn infants. This study was conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki 1999 and had the approval of 3 ethics committees (The Children's Hospital at Westmead, Westmead Hospital, and the Children, Youth and Women's Health Service, Adelaide). Written informed consent was obtained from parents/guardians before the enrollment of infants.

Neonates in group 1 received aPV at birth (within 5 days) and a second dose at 1 month of age. Those in group 2 received aPV within 5 days of birth only and those in group 3 followed the routine vaccination schedule. In Australia, this includes hepatitis B vaccine at birth and, at 2, 4, and 6 months of age, diphtheria, tetanus, pertussis, hepatitis B, and *Haemophilus influenzae* type b antigens (given in this study as DTaP-HBV-IPV/Hib vaccine (*Infanrix Hexa*) as well as 7 valent pneumococcal conjugate vaccine (*Prevnar*). Thus overall, subjects in group 1, 2, and 3 received 5, 4, and 3 doses respectively of a pertussis-containing vaccine by 6 months of age.

Subjects

Eligible subjects were healthy infants, who had completed at least 36 weeks gestation, were born after an uncomplicated pregnancy to mothers seronegative for hepatitis B surface antigen (HbsAg) and were enrolled within 120 hours of birth.

Enrollment in the study was excluded by any of the following: known contraindications to vaccination[23]; administration of immunoglobulins or blood products preceding the first dose of study vaccine or their planned administration during the study period; any confirmed or suspected immunosuppressive or immunodeficient condition in the parent or child and major congenital defects or serious chronic illness. The study was conducted in Sydney and Adelaide, Australia between February 2005 and March 2007. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN012605000013662).

Vaccines

A single dose of investigational aPV (0.5 mL) containing pertussis toxin (PT) 25 μg, pertactin (PRN) 8 μg, filamentous hemagglutinin (FHA) 25 μg, and 0.5 mg aluminum as hydroxide salts was supplied by GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium. All infants received 10 μg hepatitis B surface antigen (HbsAg) with 0.25 mg aluminum hydroxide adjuvant (*Engerix B*). The aPV was administered intramuscularly into the right anterolateral thigh and the HBV vaccine into the left anterolateral thigh concomitantly in Groups 1 and 2 prior to 120 hours of age. The antigen composition of the aPV used at birth and 1 month was identical to that in the combined DTaP-HBV-IPV/Hib vaccine (*Infanrix hexa*) in routine use. As indicated above, routine scheduled vaccines at 2, 4, and 6 months included *Infanrix hexa* and 7 valent pneumococcal conjugate vaccine (*Prevnar*—Wyeth pharmaceuticals), whose composition is listed elsewhere.[9] *Infanrix hexa* was administered intramuscularly in the right thigh and *Prevnar* in the left thigh at 2, 4, and 6 months of age by study nurses.

Assessment of Immunogenicity

In total, 5 blood samples were collected. To reduce the number of blood samples required from the infant, the first sample was obtained from the mother at the same time as the infant received the first vaccination (Pa and HBV or HBV alone). Subsequent samples (n = 4) were collected from infants at 2, 4, 6, and 8 months of age. Samples were centrifuged, serum separated, stored at −80°C and shipped frozen to GSK Biologicals, Belgium (GSK) where all serologic assays were performed.

Pertussis toxin (anti-PT), pertactin (anti-PRN), and filamentous hemagglutinin (anti-FHA) IgG antibody concentrations were measured at each sampling point by enzyme linked immunosorbent assay (ELISA: cut-off 5 EL.U/mL), using standard assay methods at the GSK laboratory developed for licensure of DTPa vaccines.

Antidiphtheria (cut-off 0.1 IU/mL), antitetanus (cut-off 0.1 IU/mL), and anti-PRP (cut-off 0.15 μg.mL) IgG antibodies were measured by ELISA on the sample taken at 8 months of age (2 months after the final vaccine dose). Hepatitis B surface antibodies (anti-HBs) were measured by ELISA (AUSAB, Abbott Laboratories) as per the manufacturer's recommendations (cut-off 10 mIU/mL) on samples collected at 8 months of age. The laboratory was blind to the study assignment of subjects. There was no formal surveillance for pertussis infection.

Assessment of Reactogenicity

After administration of each vaccine, all infants were observed for 30 minutes. Vaccine reactogenicity and safety was assessed using a 7 day diary card after each vaccination. Parents were given a thermometer, instructed in its use, and asked to record temperature and any solicited adverse reactions 3 and 6 hours after injection and at bedtime each evening for 7 days. Solicited adverse reactions included: fever, drowsiness (unusually sleepy or inactive), irritability, anorexia, vomiting, redness, and swelling at the vaccination site (each measured in millimeters) and pain. All unsolicited adverse events occurring within the time interval between vaccinations were recorded by parent/guardian and/or study physician at each study visit. Telephone contact was made with parents/guardians on days 2 and 7 to enquire about adverse events and encourage completion of the diary cards following vaccination. The total duration of safety follow-up was 2 months following the final vaccine dose at 6 months. Any serious adverse event, including hospitalization, was assessed by an independent vaccine safety committee.

Statistical Analysis

The investigators were responsible for study design and conduct and performed all statistical analyses on individual patient data. Only subjects who had completed the vaccine schedule according to protocol and had at least 2 assay results available, including the maternal baseline sample, were included in the immunogenicity analysis. For pertussis antigens, antibody geometric mean concentrations (GMC) with 95% confidence intervals (CI) were calculated from the antilog of the mean of the log transformed values. Values below the laboratory assay cut-off were assigned a value half of the cut-off value to calculate the GMC.

The primary objective of the study was to assess if IgG antibody to PT and PRN was significantly higher in group 1 at 2 months of age (after 2 aPV doses) than after 1 dose in group 2 and no prior doses of pertussis-containing vaccine in group 3. As no universally agreed serologic correlate of protection exists for pertussis, serologic response, defined as a 4-fold increase from the prevaccination antibody titer, was examined as the variable of interest. For diphtheria, tetanus, Hib, and hepatitis B, serologic response was defined as any level above the lower limit for detection in the assay used for each antibody (0.1 IU/mL, 0.1 IU/mL, 0.15 μg/mL, and 10 mIU/mL, respectively). Comparisons of antibody responses between groups were using log-transformed data by the independent samples t test with $P < 0.05$ indicating a possible group difference. The proportion of study group subjects

with a serological response and local and systemic reactions after vaccination in study groups were compared by Fisher exact test.

To detect a significant difference for the primary outcome of detectable antibody after the second dose, and to allow for drop-outs and failure to obtain some specimens by venipuncture, we aimed to recruit 25 subjects per arm for this pilot study. Our sample size calculations had indicated that this number of subjects would give 80% power to detect a 50% difference in the proportion of infants achieving detectable PT antibody.

RESULTS

We enrolled 76 eligible newborns from February 2005 to June 2006. The mean gestational age was 39.8 weeks, 59% were male and there was no significant difference in birth weight between groups. (Table 1) Sixty-eight infants remained enrolled to completion of the vaccination schedule at 6 months and 64 infants until the completion of safety follow-up at 8 months. Eight infants, 2 from Group 1, 1 from Group 2, and 5 from Group 3 withdrew from the study after enrollment and before the first blood sample at 2 months for varied reasons including relocation (1), declining blood tests (4), and inadvertent vaccination with non study vaccines (3).

Immunogenicity

Antibody Responses to Pertussis Vaccination

At enrolment, the GMC of maternal IgG to both pertussis toxin (PT) and pertactin (PRN) was not significantly different among groups. However, infants randomized to group 2 had significantly higher maternal anti-PT IgG than those randomized to group 3 (GMC 6.2 vs. 3.3, $P = 0.04$).

With respect to GMCs, at 2 months, following 2 doses of aPV, Group 1 infants had statistically significantly higher GMCs for anti-PT, anti-FHA and anti-PRN IgG compared with both Group 2 and 3 infants (Tables, Supplemental Digital Content 1, 2, and 3, http://links.lww.com/INF/A249, http://links.lww.com/INF/A250, and http://links.lww.com/INF/A251). For anti-PT IgG, levels remained significantly higher in group 1 compared with groups 2 and 3 at 4 and 6 months (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A249) but not at 8 months, with little increase in IgG PT seen after a fourth or fifth dose. For anti-PRN IgG, at 4 months of age, after 3 doses of a pertussis–containing vaccine, levels were significantly higher in group 1 compared with groups 2 (2 doses) and 3 (1 dose) and although in contrast to PT, IgG to PRN increased with each successive dose of pertussis-containing vaccine, differences at 6 or 8 months of age were no longer statistically significant. (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A250). For anti-FHA, levels were significantly higher in groups 1 and 2 compared with group 3 at 4 months of age. (Table, Supplemental Digital Content 3, http://links.lww.com/INF/A251).

With respect to the proportion above the limit of detection, at 2 months old, after 2 doses of a pertussis – containing vaccine, 88% of group 1 infants had a level of IgG to PT above 5 EU/mL compared with 43% of those in group 2 (1 dose) and 15% of group 3 (no doses). Similarly, all group 1 infants had detectable antibody (>5 U/mL) to PRN 1 month after the second dose of Pa at 2 months, compared with 33% for those in group 2 who had received a dose at birth only and 30% for controls. Significantly more infants in group 1 had a 4-fold rise in anti-PT IgG from maternal values to 2 months old (56% vs. 5 and 0% respectively for groups 2 and 3, $P < 0.02$).

There was no evidence of later hypo-responsiveness to pertussis antigens in infants who received Pa vaccine within 5 days of birth. Pertussis antibody levels from 4 months to 8 months of age converged between groups, particularly for PT, and at 8 months did not significantly differ from control infants (Fig. 1).

Influence of Maternal Pertussis Antibody Levels at Birth

At 2 months of age, antibody levels in groups 2 and 3 were slightly lower than maternal levels, consistent with loss of maternal antibodies. Of the 8 infants in Group 1 who had detectable anti-PT IgG in maternal sera (>5 EL.U/mL), 6 (75%) showed an increase in IgG PT between birth and 2 months of age compared with 1 (7%) of the infants in groups 2 and 3 combined who had detectable maternal antibody. At 8 months of age, the GMC for anti-PT and anti-PRN IgG among infants in groups 1, 2, and 3 whose mothers had detectable IgG was similar to infants in each of the 3 groups whose mothers had no detectable IgG antibodies to these antigens. However, when groups were combined after 3 doses, significantly lower anti-PRN and anti-FHA levels were found in those with detectable maternal antibody at baseline (Table 2).

Antibody Responses to Other Vaccine Antigens

Two months after completion of the primary immunization schedule, 100% of subjects in all groups had IgG levels to diphtheria and tetanus above those usually associated with protection (0.1 U/mL), with no significant difference between the groups (Table 3). There was a nonsignificant trend to reduced hepatitis B surface antibody GMC responses in infants who received the Pa vaccine at birth (Group 1 and 2 vs. Group 3), however all were above the anti-HBs level associated with protection (10 mIU/mL). Similarly, Group 1 infants had nonsignificantly lower GMCs against Hib and a lower proportion with anti PRP IgG above 1 μg/mL, compared with Group 2 and 3 infants (26% vs. 45% vs. 47%; Table 3). Infants in groups 1 and 2 who had a 4-fold increase in anti-PT level from baseline to 4 months old had nonsignificantly higher Hib and hepatitis B surface antibody levels at 8 months compared with those with less than a 4-fold rise.

Reactogenicity

Birth aPV was well tolerated, with no vaccine-related severe adverse events detected. After the birth dose, only 2 infants had redness or swelling >10 mm and none had fever >38C. Following the 6 month vaccination, there was no difference in the proportion

TABLE 1. Characteristics of Study Subjects According to Group

Enrolled Subjects	Group 1 n=27	Group 2 n=23	Group 3 n=26
Mean birthweight (g) (range)	3454 (2840–4215)	3306 (2575–4205)	3560 (2600–4370)
Mean gestation weeks (range)	39.8 (38–41.3)	39.4 (37.2–41.3)	39.7 (37–41.5)
% Male (n)	68% (17)	55% (12)	55% (12)
% Vaccinated day 0–2 (n)	36% (9)	50% (11)	n/a
% Vaccinated day 3–5 (n)	64% (16)	50% (11)	n/a
Withdrew prior to 2 months old	2	1	5

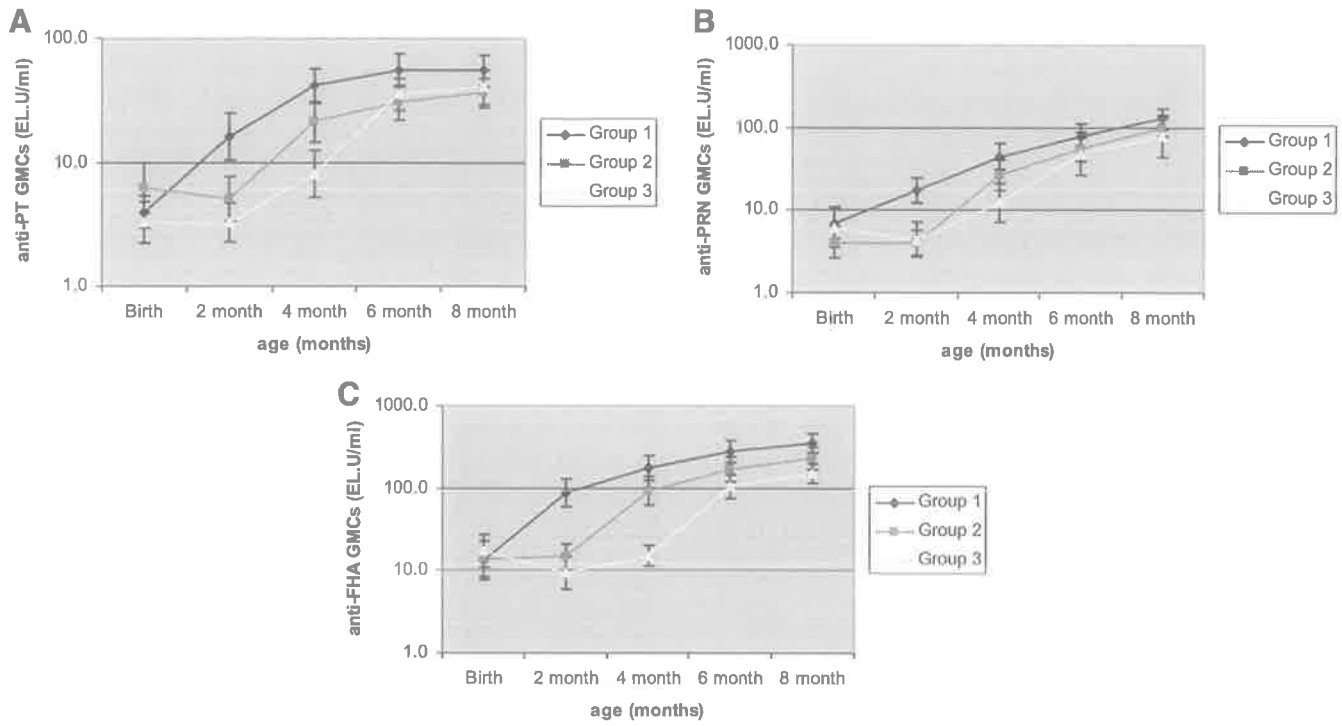

FIGURE 1. Anti-pertussis antibody geometric mean concentrations (GMCs) from birth until 2 months after completion of primary vaccination. A, Antibody response to pertussis toxin according to group and age; B, antibody response to pertactin according to group and age; C, antibody response to filamentous haemagglutinin according to group and age.

TABLE 2. Pertussis Antibody Responses After 3 Doses for Combined Group (1, 2, 3)* According to Detectable or Nondetectable Maternal Antibody at Baseline

Baseline Pertussis Antibody	Maternal Antibody Detectable (>5 EL.U/mL)		Maternal Antibody Not Detectable (<5 EL.U/mL)	
	N	GMC† (95% CI)	N	GMC† (95% CI)
Anti-PT	18	31.8 (21.9–55.2)	42	39.5 (32.1–48.7)
Anti-PRN	25	36.4‡ (24.0–55.2)	35	78.9‡ (61.7–100.8)
Anti-FHA	49	151.2† (123.8–184.6)	11	253.5† (187.2–343.4)

*Combined groups after 3 doses:
(a) Antibody responses after 3 doses (group 1–aged 4 mo, group 2–aged 6 mo, group 3–aged 8 mo) in those with detectable maternal antibody were combined.
(b) Antibody responses after 3 doses (group 1–aged 4 mo, group 2–aged 6 mo, group 3–aged 8 mo) in those with no detectable maternal antibody were combined.
†Anti-FHA GMC significantly different between combined groups after 3 doses for detectable maternal antibody vs. non detectable antibody. ($P = 0.02$).
‡Anti-PRN GMC significantly different between combined groups after 3 doses for detectable maternal antibody vs. non detectable antibody. ($P = 0.002$).
GMC indicates geometric mean concentration (EL.U/mL).

of infants with swelling or redness >10 mm between group 1 (after 5 doses, 17% [n = 4]), group 2 (after 4 doses, 14% [n = 3]) or group 3 (after 3 doses, 22% [n=4]) ($P > 0.5$). Similarly, the proportion with reported systemic reactions or fever was similar between the groups. Two infants required hospitalization for pyloric stenosis, one aged 4 weeks in Group 2 and the other aged 6 weeks in group 3.

Pertussis Infection

One male infant in group 1 who had received 3 doses of aPV (birth, 1 month and 2 months of age) developed symptoms of mild fever, cough and rhinorrhea at 115 days, 30 days after the third dose. Pertussis was identified by PCR from a nasopharyngeal aspirate on day 134 but pertussis culture was negative. A maternal aunt had a cough consistent with pertussis commencing approximately 14 days before onset of symptoms in the infant, with positive single titer serology. This infant had a mild clinical course and did not require hospital admission. All antipertussis antibodies at 2 months of age, measured after 2 doses and 30 days before onset of symptoms were detectable (anti-PT 15 EU/mL, anti-FHA 198 EU/mL, and anti-PRN 39 EU/mL). Convalescent antipertussis antibodies at 4 months (after 3 doses of aPV and 11 days post diagnosis of infection) increased 2-fold for anti-PT and anti-PRN and nearly 2-fold for anti-FHA. Antipertussis antibody values decreased from 6 months to 8 months after the fifth dose of an acellular pertussis-containing combination vaccine.

DISCUSSION

This is the first study to assess the immunogenicity and reactogenicity of 2 doses of aPV (birth and 1 month) given before

TABLE 3. Immune Responses 2 Months After Completion of Primary Vaccination for Concomitant Antigens According to Group

Antibody	Threshold	Group 1*			Group 2*			Group 3*		
		Number†	% > Threshold	GMC‡ (95% CI)	Number†	% > Threshold	GMC‡ (95% CI)	Number†	% > Threshold	GMC‡ (95% CI)
Hepatitis B	>10 mIU/mL	20	100	292.9 (14.2–604.1)	19	100	540.5 (301.8–967.8)	15	100	821.8 (488.2–1383.3)
	>100 mIU/mL		80.0			95.0			100	
Haemophilus influenzae b	>0.15 μg/mL	23	65.2	0.39 (0.2–0.75)	20	95.0	1.03 (0.47–2.22)	19	89.5	0.8 (0.41–1.58)
	>1 μg/mL		26.0			45.0			47.4	
Diphtheria	>0.1 IU/mL	23	100	1.64 (1.2–2.24)	20	100	1.7 (1.18–2.46)	19	100	1.97 (1.3–2.98)
	>1 IU/mL		78.2			75.0			84.0	
Tetanus	>0.1 IU/mL	23	100	0.84 (0.55–1.28)	20	100	1.46 (0.98–2.17)	19	100	1.34 (0.89–2.04)
	>1 IU/mL		47.8			55.0			78.9	

*Group 1—Pa vaccine at birth and one month then Infanrix Hexa at 2, 4, and 6 mo of age.
*Group 2—Pa vaccine at birth then Infanrix Hexa at 2, 4, and 6 mo of age.
*Group 3—Infanrix Hexa at 2, 4, and 6 mo of age.
†Number—according to protocol number of subjects who had blood sample collected at 8 mo old for antibody measurement.
‡GMC indicates geometric mean concentration.

2 months of age. The study is also unique in that all infants received HBV vaccine at birth, thus allowing direct comparison of the potential influence of birth aPV on concomitant HBV vaccine responses.

Despite its small sample size, this study showed statistically significantly higher GMCs of anti-PT, anti-PRN and anti-FHA IgG antibody at 2 months of age in infants who received aPV at birth and 1 month of age, compared with both those receiving aPV at birth only and those who had not been vaccinated. The titers of anti PT and anti PRN IgG achieved after 3 doses of acellular pertussis-containing vaccine (birth, 1 and 2 months of age) were similar to those seen with 3 doses administered at 0, 2, 4 or the conventional 2, 4, and 6 months of age. This raises the prospect of achieving protection, particularly against severe pertussis, at least 4 months earlier than under current vaccination schedules, subject to the caveat that antibody correlates of protection against pertussis disease of different severities in infants have not been clearly established. Observational studies suggest some protection against severe pertussis from even 1 dose of vaccine, possibly due to rapid antibody production following natural exposure in a primed infant.[4,5] In Germany, estimated vaccine effectiveness against infant hospitalization was 68% after the first and >90% after the second dose of DTPa.[4] In Sweden, the incidence of pertussis fell from 230 to 235 (cases per 100,000 person years) after no or 1 dose of pertussis vaccine to 52 after 2 doses.[5] Our study also suggests that a first dose at birth primes the immune system, with a significant increase in antibody after the second dose, whether given at 1 or 2 months of age.

Four doses of acellular pertussis-containing vaccines within 4 months of birth was not associated with any major local or systemic adverse events in this small number of subjects. Similar to other studies, monovalent aPV given at birth was well tolerated with no increase in reactogenicity identified at birth or following later vaccine doses compared with infants receiving the routine vaccine schedule.[20–22] One participant, who had received 3 doses of a pertussis containing vaccine (0, 1, 2 months), developed laboratory-proven pertussis infection at 3 months of age. The illness was clinically mild and may not have been detected outside the clinical trial setting. Symptoms may have been substantially attenuated by vaccination, although pertussis infection is not universally severe in infants, and infection occurred despite documented prior antibody responses to pertussis antigens.

There are some differences in the antibody responses in our study compared with 3 other recent studies which examined administration of differing acellular pertussis- containing vaccines at birth. The study most similar to ours, which was conducted in Germany using aPV produced by the same manufacturer (Glaxo-SmithKline) and the same laboratory for antibody measurement, also demonstrated a significantly higher GMC of antipertussis IgG to PT, PRN, and FHA in infants after 2 doses of Pa at birth and 2 months compared with controls, with no subsequent reduction in antibody response.[22] In an earlier Italian study, where a aPV manufactured by Chiron was given at birth and 3 months of age, higher PT IgG were also seen in these infants at 5 months.[20] By contrast, a recently reported study conducted in the United States, where a DTaP vaccine manufactured by Sanofi Pasteur was given at birth, found GMCs for both PT and FHA IgG post completion of primary vaccination were lower in the experimental group than in controls.[21] This may be related to the different composition of the pertussis antigens in the GSK (3 component) and Sanofi Pasteur (5 component) vaccine, an effect of concomitant diphtheria and tetanus toxoid or some other factor. Hyporesponsiveness, a concern of early studies[16] was not seen in our study or in Germany, with equivalent antipertussis antibody titers at 8 months with or

without a birth dose, however antibody titers converged between groups by 8 months old. This may relate to a biologic feedback phenomenon of achieving a "ceiling" of antibody level designed to protect the body from immune overload due to excessive antibody production. However, the US[21] and Italian[20] studies found that infants who received a pertussis-containing vaccine at birth had lower PT IgG at 7 to 8 months of age. In particular, the US study[21] found that the significantly lower pertussis antibody titers in infants who received DTaP at birth documented at 8 months persisted to 18 months of age, which they postulated may be due to the combination of diphtheria, tetanus, and aP in the combination vaccine resulting in interference with antigen presentation or B lymphocyte priming.

Maternal antibodies to pertussis can interfere with subsequent infant responses.[13,14,24] In our study, a small impact of maternal pertussis antibody was found when groups were combined, but this has not been adequately evaluated, particularly with respect to higher titers of maternal antibody, as our sample size was small and few mothers had detectable antibody. Larger studies, especially among women with higher pertussis antibody titers, such as would be expected following receipt of pertussis-containing vaccine as adolescents or adults or following recent natural infection, are needed. With increasing use of adult acellular pertussis booster vaccines in many countries, the potential for impact of higher maternal antibodies on infant pertussis disease and/or infant responses to pertussis-containing vaccines will become a more important issue.[3]

Other antigens included with pertussis antigens in combination vaccines include diphtheria, tetanus, polio, hepatitis B and *H. influenzae* type b (Hib). Vaccines given concomitantly in recommended national schedules in developed countries include pneumococcal conjugate and rotavirus vaccines. In the US study, infants who had received DTaP at birth had significantly lower antibody titers to diphtheria and pneumococcal serotype 14 than controls at 7 months old.[21] In the German study, attainment of anti-PRP IgG antibody responses consistent with short-term protection (>0.15 μg/mL) was significantly less after the first 3 doses (88% vs. 98%).[22] In our study, anti-PRP IgG appeared to be lower only in infants who received 2 doses of aPV before 2 months of age but power to detect any difference was low. Reduced anti-PRP IgG responses have been associated with DTaP-Hib combination vaccines, but this has only emerged as a clinical problem in one country, the United Kingdom, leading to introduction of a Hib booster.[25] Any such phenomenon following the primary series of vaccination might not be clinically relevant if a booster is routinely given. There was no significant difference in response to diphtheria and tetanus antibody responses. We did not measure responses to polio or pneumococcal antigens, but no significant differences in response to any of 3 polio serotypes were found by the only study measuring them following aPV at birth.[22] Hepatitis B vaccine (HBV) was given only to the control group in the German study,[22] whereas in our study, similar to routine practice in the US and as recommended by WHO, all participants received HBV vaccine at birth. Although reduced anti HBs antibody GMC was seen in infants receiving aPV at birth, all participants achieved protective titers (anti-Hbs >10 mIU/mL) at 8 months of age.

This study had several limitations including, small sample size, lack of data on response to all concomitant antigens (polio and pneumococcal serotypes) and has not examined persistence of antibody beyond 8 months of age.

In total, 202 infants have received monovalent aPV or DTaP vaccine at birth in recent published studies.[20–22] Despite the varying immunogenicity data referred to above, no severe adverse events have been reported. The possibility of later reductions in antibody response, and/or interference with responses to concomitantly administered antigens, necessitates larger studies. These include the timing of the second dose of pertussis-containing vaccine. A second dose at 6 weeks of age would be feasible and practical, as current combination vaccines including acellular pertussis antigens are licensed from this age and 6 weeks is consistent with the current WHO schedule. If pertussis vaccine given at birth was included in the WHO Expanded Program on Immunization schedule, infants would then receive 3 doses of a pertussis-containing vaccine by 10 weeks of age (0, 6, 10 weeks). At present, most developing countries use whole cell pertussis (Pw) vaccine in combination with diphtheria and tetanus in the primary immunization schedule and no recent data exist about the immunogenicity and reactogenicity of Pw alone at birth. Future studies with larger samples sizes are needed to address several important issues including more precise estimates of the occurrence of adverse reactions, including the magnitude of any bystander interference with responses to concomitant antigens[26] and the influence of higher levels of maternal antibodies on infant pertussis responses.

Nearly 3 quarters of a century ago, studies attempted pertussis vaccination at birth and in pregnant women, to prevent pertussis in early infancy.[15,17] Current global epidemiologic data indicate that pertussis remains a significant problem in early infancy and new strategies are needed.[27] The availability of acellular pertussis vaccines, with reduced reactogenicity, has led to renewed interest in neonatal pertussis vaccination and in maternal vaccination during pregnancy.[28,29] With respect to neonatal pertussis vaccination strategies, these antibody response data suggest that potentially protective antibody can be achieved before 2 months of age and that no more than 4 doses before 6 months of age are necessary. Larger and more detailed neonatal vaccine studies are needed to evaluate the potential of this approach to prevent death and morbidity from pertussis disease in infants under 3 months of age.

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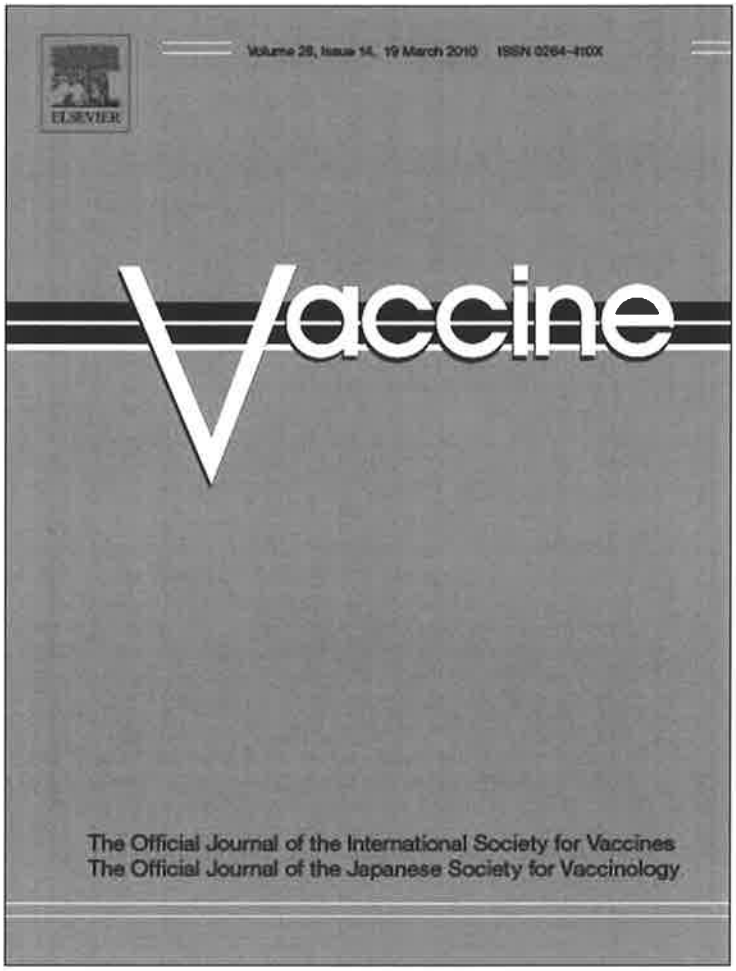

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Th2-polarisation of cellular immune memory to neonatal pertussis vaccination

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ABSTRACT

Current infant vaccination against pertussis in North America and Australia requires three doses of vaccines including diphtheria, tetanus and acellular pertussis antigens (DTaP) at 2, 4 and 6 months of age. Interest is growing in the possibility that vaccination at birth might provide earlier protection of infants, but early vaccination also gives rise to concerns over the potential for excessive Th2-polarisation of pertussis-specific T-cell memory profiles. We evaluated this issue as part of a small pilot study comparing infants receiving a monovalent acellular pertussis vaccine (aP) at birth or birth and at 1 month, followed by DTaP at 2, 4 and 6 months with infants receiving DTaP only from 2 months. We compared *in vitro* Th-memory responses at 8 months and pertussis-specific IgG in serum at 2, 4, 6 and 8 months. Neonatal vaccination elicited earlier IgG responses, but accompanying Th-memory profiles displayed a strong Th2 bias with high IL-5 and IL-13 production. The correlation between T-cell memory profiles and other clinical outcomes should be evaluated in larger trials of neonatal aP vaccine.

1. Introduction

Acellular pertussis (aP) vaccines, in combination with diphtheria and tetanus (DTaP) have been used in the national immunisation program in Australia since 1997, with significant improvements in immunisation coverage compared with whole cell pertussis vaccines (DTPw) [1]. Despite this, infants under 6 months of age continue to have the highest annual notification rates of hospitalisation and death [2]. During the period from birth to the first pertussis-containing vaccine at 2 months of age, newborns are more susceptible to pertussis infection due to the relatively immature state of their immune system [3], with lack of cellular immunity against pertussis antigens, and the inadequacy of antibody-mediated protection from at best modest levels of maternally derived IgG antibodies [4,5]. A neonatal pertussis immunisation strategy that could significantly redress these immunological deficiencies would potentially provide newborns with a significantly greater level of early protection against pertussis disease than currently available, and there is intense interest internationally in developing this approach.

In Australia, Hepatitis B (Hep B) vaccine is routinely given at birth and has been proven to be safe and effective in this age group [6]. Similarly, in many countries, BCG vaccine is routinely administered at birth and induces strong cellular immune memory responses [7], in particular Type-1-memory associated with production of cytokines which mediate sterilising immunity such as interferon gamma. This demonstrates the general principle that neonates are capable of responding effectively to at least some types of vaccines. In addition, previous studies of neonatal immunisation have shown monovalent aP vaccine to be safe and have established that it is possible to induce early humoral immune responses to pertussis antigens [8–10]. However, theoretical concerns remain regarding qualitative aspects of vaccine immunity induced in neonates due to the intrinsically Type-2-polarised nature of immune responses (default to production of IL-4, IL-5 and IL-13) in this age group, which have the potential to antagonise development of Type-1-dependent sterilising immunity [3]. For example, studies of RSV infection in both neonatal mice [11] and human neonates [12] concluded that early infection commits the immune system to development of strong primary Type-2 immunity, and moreover this may influence symptomatology associated with future infections via the presence of an excessive component of pro-inflammatory Type-2 cytokines in the resultant memory response [11]. Such concerns are also relevant to acellular pertussis vaccines, which lack intrinsic Th1-stimulatory components and/or contain Th2-stimulatory agents, but this issue has not yet been

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systematically addressed in the context of neonatal pertussis vaccination.

As an initial step in this direction, we present the results from a pilot study comparing administration of monovalent pertussis vaccine at birth with the Australian standard vaccination schedule [13], where the first pertussis-containing vaccine is given at 2 months after birth. We evaluated cell-mediated immunity in a sub-set of participants in this pilot study; data on antibody responses for all study participants are presented elsewhere [14].

2. Materials and methods

2.1. Subjects and vaccines

From a pilot study of 76 healthy newborns recruited at The Children's Hospital Westmead, Sydney and The Women's and Children's Hospital, Adelaide, 62 consented to an add-on study of cell-mediated immunity to vaccine antigens, requiring that additional blood be collected at 8 months of age. Of these, 30 subjects had blood samples of sufficient volume to be included in this sub-study.

One group of subjects (Group 3 herein; $n = 10$) received vaccines according to the Australian national immunisation program [13], which is Hepatitis B vaccine (Engerix B®, GlaxoSmithKline [GSK] Biologicals, Rixensart, Belgium) at birth, followed by a combination Diphtheria, Tetanus, acellular Pertussis, Hepatitis B, Inactivated Polio Virus, *Haemophilus influenzae* type b vaccine (DTaP-HBV-IPV/Hib, Infanrix®-Hexa, GSK) and 7-valent pneumococcal conjugate vaccine (Prevenar®, Wyeth Pharmaceuticals Inc., Philadelphia, USA) at 2, 4 and 6 months of age. Two other groups in addition were given investigational monovalent acellular pertussis vaccine (aP) vaccine (containing 25 μg PT, 25 μg FHA, 8 μg PRN, and 0.5 mg aluminium as hydroxide salts, GSK), either as a single dose at birth (Group 2; $n = 11$) or at birth and at 1 month of age (Group 1; $n = 9$). Prior to immunisation, peripheral blood was obtained at 2, 4, 6, and 8 months of age. Maternal blood was obtained at birth. This study was carried out with the approval of relevant institutional ethics committees.

2.2. Assessing humoral responses

Blood samples collected from infants at each time point were centrifuged, serum separated and stored at −80 °C for analysis by GSK Biologicals, Rixensart, Belgium. An ELISA method was used to determine geometric mean concentrations (GMC) of IgG specific for pertussis toxoid (PT), filamentous haemagglutinin (FHA) and pertactin (PRN), as per GSK standard assays developed for licensure of DTaP vaccines (cut off: 5 EL U/ml).

2.3. Assessing cellular responses

To measure the levels of cytokines produced *in vitro* in response to vaccine antigen stimulus, blood was collected at 8 months into an equal volume of RPMI 1640 (Cytosystems, Castle Hill, Australia) containing preservative-free heparin and processed within 24 h of collection employing standard methodology, which does not significantly alter subsequent *in vitro* cellular responses [15,16]. Briefly, peripheral blood mononuclear cells (PBMC) were isolated by ficoll density gradient centrifugation. Cells were washed twice in RPMI 1640 containing 2% foetal calf serum, and counted using white cell counting fluid (crystal violet in 2% acetic acid and sodium chloride). Cells were then resuspended in freezing medium (10% DMSO in RPMI 1640) and cryopreserved in liquid nitrogen.

As described elsewhere [17], cryopreserved PBMC were batch analysed in groups of 8 within a short period and with identical reagents. Aliquots of 0.5×10^6 cells were cultured in duplicate wells

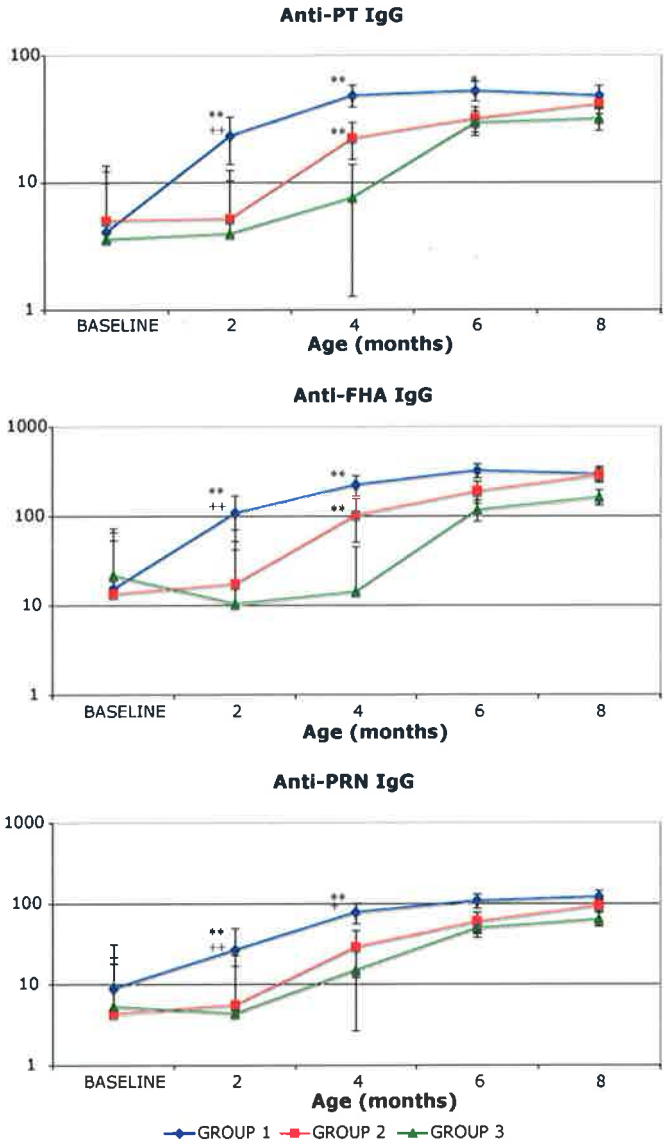

Fig. 1. Vaccine-specific IgG antibody production measured in serum samples from 30 children at 2, 4, 6, and 8 months. BASELINE: maternal antibody titre; Group 1: aP at birth and 1 month plus DTaP at 2, 4, and 6 months; Group 2: aP at birth plus DTaP at 2, 4, and 6 months; Group 3: DTaP at 2, 4, and 6 months. Data are shown as geometric mean concentration (EL U/ml), with standard error bars. *Significant at the 95% level when comparing Group 1 or Group 2 with Group 3. **Significant at the 99% level when comparing Group 1 or Group 2 with Group 3. ⁺Significant at the 95% level when comparing Group 1 with Group 2. ⁺⁺Significant at the 99% level when comparing Group 1 with Group 2.

for 96 h alone or together with; 1 μg/ml PT + 1 μg/ml FHA + 1 μg/ml PRN (Mix, all generously supplied by GSK), or 1 μg/ml PT, or 1 μg/ml FHA, or 1 μg/ml PRN, or 0.5 Lf/ml tetanus toxoid (TT, CSL, Melbourne, Australia), or 1.0 Lf/ml diphtheria toxoid (DT, CSL), or 2.5 μg/ml Hepatitis B surface antigen (ProSpec-Tany TechnoGene Ltd., Rehovot, Israel). Antigen concentrations employed were based on preliminary dose response experiments.

The levels of IL-5, IL-6, IL-13 and IFNγ in culture supernatants were measured by in-house time-resolved fluorometry assays as described previously [4,18]. The cytokine values for each stimulus are displayed in picograms per millilitre (pg/ml). The limit of detection for these assays was 10 pg/ml for each cytokine. Significant differences shown between the groups were determined by Mann–Whitney *U*-test for unpaired responses using SPSS software package (SPSS Inc., Chicago, USA).

Table 1
Cytokine responses to *in vitro* stimulation of PBMC with vaccine and control antigens at 8 months of age.

Stimulus	Group	IL-5	IL-13	IFNγ	IL-6
Unstimulated	1	0 (0, 0)	0 (0, 12)	0 (0, 0)	25 (0, 121)
	2	0 (0, 17)	0 (0, 29)	0 (0, 9)	11 (0, 600)
	1,2	0 (0, 17)	0 (0, 29)	0 (0, 9)	22 (0, 600)
	3	0 (0, 24)	0 (0, 9)	0 (0, 0)	27 (0, 359)
Mix (PT, FHA, PRN)	1	493 (0, 1008)[0.010] *[0.200]*	460 (0, 1220)[0.013] *[0.260]*	8 (0, 92) [n.s.]	394 (0, 1587) [n.s.]
	2	137 (0, 492) [0.003] *[0.060]*	199 (0, 652) [0.004] *[0.080]*	15 (0, 347) [n.s.]	218 (15, 1422) [n.s.]
	1,2	163 (0, 1008)[0.001] *[0.020]*	220 (0, 1220)[0.001] *[0.020]*	11 (0, 347) [n.s.]	244 (0, 1587) [n.s.]
	3	0 (0, 55)	23 (0, 108)	8 (0, 32)	108 (0, 1990)
PT	1	30 (0, 421) [n.s.]	63 (0, 244) [n.s.]	0 (0, 211) [n.s.]	56 (11, 353) [n.s.]
	2	32 (0, 145) [0.016]	59 (0, 240) [0.043]	0 (0, 21) [n.s.]	36 (11, 1054) [n.s.]
	1,2	30 (0, 421) [0.023]	60 (0, 244) [0.043]	0 (0, 211) [n.s.]	42 (11, 1054) [n.s.]
	3	0 (0, 18)	9 (0, 57)	3 (0, 25)	22 (0, 158)
FHA	1	61 (0, 46) [n.s.]	137 (0, 464) [n.s.]	59 (0, 475)[0.014]	104 (0, 5106) [n.s.]
	2	65 (0, 553) [0.021]	89 (0, 699) [0.012]	16 (0, 79) [n.s.]	47 (10, 314) [n.s.]
	1,2	63 (0, 553) [0.013]	102 (0, 699) [0.009]	37 (0, 475)[0.027]	53 (0, 5106) [n.s.]
	3	9 (0, 32)	7 (0, 48)	0 (0, 34)	32 (0, 423)
PRN	1	0 (0, 9) [n.s.]	7 (0, 29) [n.s.]	0 (0, 0) [n.s.]	182 (18, 608) [n.s.]
	2	0 (0, 0) [n.s.]	0 (0, 21) [n.s.]	0 (0, 0) [n.s.]	190 (27, 1877) [n.s.]
	1,2	0 (0, 9) [n.s.]	0 (0, 29) [n.s.]	0 (0, 0) [n.s.]	186 (18, 1877) [n.s.]
	3	0 (0, 40)	0 (0, 0)	0 (0, 0)	29 (0, 425)
TT	1	0 (0, 0)	0 (0, 8)	0 (0, 0)	72 (22, 418)
	2	0 (0, 76)	11 (0, 107)	0 (0, 47)	166 (13, 756)
	1,2	0 (0, 76)	0 (0, 107)	0 (0, 47)	109 (13, 756)
	3	0 (0, 26)	4 (0, 49)	0 (0, 25)	29 (18, 278)
DT	1	0 (0, 23)	7 (0, 24)	0 (0, 0)	3726 (1615, 10,350)
	2	9 (0, 135)	26 (0, 162)	8 (0, 17)	5171 (2951, 13,962)
	1,2	4 (0, 135)	14 (0, 162)	0 (0, 17)	5151 (1615, 13,962)
	3	7 (0, 40)	31 (0, 102)	0 (0, 28)	4321 (1664, 7481)
HB	1	0 (0, 14)	25 (8, 31)	276 (269, 278)	4262 (4251, 27,963)
	2	0 (0, 49)	19 (10, 87)	240 (144, 1258)	12304 (5121, 32987)
	1,2	0 (0, 49)	19 (8, 87)	269 (144, 1258)	11,989 (4251, 32,987)
	3	34 (0, 278)	57 (9, 369)	210 (0, 1164)	14,573 (1858, 16,541)

Data are median cytokine concentrations in pg/ml (min, max). For pertussis antigens [uncorrected p value] derived from Mann–Whitney U-test comparing differences between either Group 1, or Group 2, or Groups 1 and 2 combined, with Group 3. *[p value following Bonferonni correction as per text].* [n.s.]: not significant at the 95% level; unstimulated: medium only; Mix: mixture of three *Bordetella pertussis* vaccine antigens, pertussis toxoid (PT), filamentous haemagglutinin (FHA) and pertactin (PRN); TT: tetanus toxoid; DT: diphtheria toxoid; HB: Hepatitis B surface antigen. No significant differences between groups were seen for TT, DT and HB responses.

3. Results

3.1. Early aP vaccination results in increased vaccine-specific IgG titres

Infants receiving 2 early doses of aP at birth and 1 month (Group 1) have significantly increased pertussis antigen specific IgG titres by 2 months of age relative to those in Group 3 receiving the standard DTaP schedule and the difference is maintained out to 6 months, or in the case of IgG anti-FHA and anti-PRN, to 4 months (Fig. 1). Vaccination-induced elevations in IgG titres in Group 2 were restricted to responses to PT and FHA and were only evident in the 4 months samples. At 2 months of age, there is a significant increase in IgG titres to all three pertussis antigens in infants receiving 2 early doses (Group 1) compared to infants receiving one early dose (Group 2), and this difference continues to 4 months in the case of PT and PRN.

3.2. Early aP vaccination results in Th2 skewed pertussis-specific cytokine responses

Pertussis-specific Th-memory responses in PBMC samples from a sub-set of the three vaccine groups after completion of respective priming schedules were assessed at the 8 months time point (Table 1). Infants who received initial aP vaccinations at birth (Groups 1 and 2) differed markedly from those receiving the standard DTaP schedule (Group 3). The key finding here relates to the cytokine production profiles in response to *in vitro* stimulation of their PBMC with the mixture of the three major pertussis vaccine antigens, which revealed increased levels of Th2 cytokines IL-5 and IL-13 (Table 1). Notably in comparison to Group 3, both IL-5 and IL-13 responses to the PT/FHA/PRN mix were significantly increased in the subjects from combined Groups 1 and 2. The level of significance in relation to this key comparison is also shown (italicised in Table 1) after application of the highly stringent Bonferroni adjustment for multiple testing, i.e. we conducted 20 independent tests (5 stimuli [Control, Mix, TT, DT, and HB] with 4 outcomes [cytokines] $\times 1$ comparison between Groups 1 and 2 versus Group 3, resulting in a $\times 20$ adjustment of p values). This finding was restricted to the Th2 cytokines and was not seen with respect to IL-6 or the Th1 cytokine IFNγ, for which no significant differences were seen between groups.

3.3. The cellular response to other antigens is not altered by early aP vaccination

We assessed the T-cell responses of the infants to Tetanus, Diphtheria and Hepatitis B antigens, and no significant differences were seen in production of IL-6, IFNγ or the Type-2 cytokines IL-5 and IL-13.

3.4. Injection site side effects

Injection site side effects (swelling > 10 mm) were detected at low frequency at the 6-month dose (Group 1, $n = 4$; Group 2, $n = 3$;

Group 3, $n = 4$) but the small sample size precludes firm conclusions being drawn based on these data.

4. Discussion

Available epidemiological evidence show that the risk of life-threatening infection from a variety of causative agents is maximal during the first 3 months of life, providing an urgent imperative for the development of vaccine protocols that provide effective protection as early as possible after birth. This challenge is already being addressed with respect to hepatitis and tuberculosis vaccines with apparent success, and an increasingly wide range of additional infectious diseases are being considered in this context [6,7]. Prominent amongst these is *Bordetella pertussis* infection, which remains a major cause of morbidity and mortality despite widespread vaccination employing the standard infant protocol in which the first priming dose of aP is given 6–8 weeks postnatally. The key issue is whether the level of protection can be improved by earlier introduction of pertussis vaccine during the neonatal period without clinically significant attendant side effects.

As noted above, the functionally immature state of the immune system during the neonatal period represents a potential impediment to the success of this approach. It is now recognised that immune function in the foetus is developmentally regulated to selectively limit capacity for generation of Th1 cytokines at the foeto–maternal interface, in order to protect the placenta against the toxic effects of these potent inflammatory agents [19]. As a consequence the balance between production of Th1 and Th2 cytokines within foetal immune responses is intrinsically skewed to favour Th2 cytokine production, and this Th2 skewing persists transiently after birth and is maximal during the neonatal period [20]. The implications of immunisation during this period with different classes of vaccines are not fully understood.

In mice, neonatal pertussis vaccination has been shown to induce pertussis-specific immune responses and to provide protection from infection [21,22]. However, whole pertussis organisms [23] and purified pertussigen (pertussis toxin) [24–26] have also been long recognised as potent Th2-selective adjuvants in experimental animals. Human studies have shown that acellular pertussis-containing DTaP vaccine induces cellular immune memory, which is strongly polarised towards the Th2 phenotype in infants [17,20,27,28] and preschoolers [29,30]. This has the theoretical potential to negatively influence bystander immune response to co-administered Th1 inducing vaccines, comparable to interference effects suggested to occur with other vaccine combinations [31]. In so doing, it may create a window period of increased risk for infection [30]. Moreover, Th2 skewed immunological memory to DTaP antigens induced by infant vaccination has been shown to increase risk for injection site reactions to subsequent booster pertussis vaccinations [30]. These findings, combined with the evidence that the likelihood of experiencing a local reaction to DTaP increases with each successive dose of the vaccine [32], raise concern that extra and early doses of DTaP-associated antigens could further increase the Th2 polarity of resultant vaccine-specific immunological memory, and hence modulate downstream host responses to antigens encountered via booster injection or natural infection. This has been shown with experimental infection with respiratory syncytial virus [11]. Although *B. pertussis* is a very different pathogen, detailed evaluation of cell-mediated as well as humoral immune responses to neonatal pertussis vaccination is clearly warranted.

In this pilot study we have contrasted pertussis-specific immune responses in infants receiving DTaP vaccine as per the standard 2–4–6 months protocol, or with additional doses of aP at birth or at birth and 1 month of age. We have assessed immunological outcomes by prospectively tracking IgG titres to three major pertussis antigens out to 8 months, and by quantifying *in vitro* Th-memory cell cytokine responses to pertussis antigens in PBMC at the 8 months time point, the latter being 1 month beyond administration of the final priming dose of DTaP. IgG antibody titres in the subgroups receiving additional aP during the neonatal period were clearly boosted above those receiving the standard DTaP regime alone, in particular in the group dosed at birth and 1 month. Of particular interest was the finding that enhanced antibody titres were evident in this latter group by 2 months of age, compared to 3 months [9], which is within the age range of maximal risk for infection. It remains to be shown whether these increased pertussis-specific IgG titres translate into reduced susceptibility to pertussis infection, but these preliminary findings provide encouragement to test this possibility in follow-up studies.

However, these potentially positive findings may be counterbalanced by findings relating to T-cell memory in the vaccinated children, if these are shown to have implications relating to safety. Notably, the cytokine balance within pertussis-specific T-cell memory in infants receiving their first aP vaccine at birth displays a clear polarisation towards significantly higher Th2 cytokine production in the form of IL-5 and IL-13, beyond the Th2 skewing already known to be a feature of the immune response to the standard DTaP regime [17,20]. Moreover, within individuals, IL-5 and IL-13 responses to the aP vaccine antigens were highly correlated (data not shown). Whether the strong Th2-polarisation observed here is due to the more extreme Th2 bias inherent in the immature immune system at birth and at 1 month of age, or to the extra vaccine dose(s) given to these two groups, could not be determined in this pilot study.

We have recently demonstrated that subsequent re-boosting of Th2-polarised memory responses primed via the standard DTaP vaccine during infancy has potential to elicit significant local side effects at the site of antigen challenge that are associated with high level production of Th2-effector cytokines such as IL-5 [20]. Given our demonstration here that neonatal aP vaccination further enhances the Th2 polarity of pertussis-specific memory responses beyond that seen with the standard infant DTaP regime, possible effects related to IgE and IL-5 following re-exposure of neonatally primed children to pertussis antigen, via vaccination or natural infection, should be considered in the safety assessment as part of follow-up studies. It is also pertinent to note that earlier studies carried out here [20] at the time of initial introduction of DTaP into the Australian standard vaccination schedule indicated that the degree of Type-2 polarisation of ensuing vaccine-specific memory was most marked in children with a positive family history of atopy. However, the Australian schedule for pertussis now does not include a booster dose until 4 years of age (previously 18 months) so this effect may be ameliorated to some extent, as suggested by surveillance data showing a steep fall in the reporting of severe local reactions. Nonetheless, we believe that formal follow-up studies of this potentially high-risk subgroup are needed.

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Developmental immunology and vaccines

A comparatively small number of studies have assessed the safety, immunogenicity, efficacy and duration of immune responses in preterm infants compared with term infants for routinely recommended childhood immunizations.

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CONTENTS

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Immune responses to vaccines in premature infants

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Immunization is one of the most significant public health interventions of the past 100 years. Each year, vaccines prevent up to three million deaths [1] and many more children are saved from morbidity and permanent disability. Although significant advances have been made in relation to knowledge of the immunogenicity, safety and effectiveness of vaccines, less is known about the immune responses of an important subset of children, that is, those born prematurely.

Recent concerns regarding the immune response of preterm infants to routine immunization schedules were raised following the introduction of the *Haemophilus influenzae* type b (Hib) conjugate vaccine for immunization in the UK in 1992. Prematurity was noted to be a risk factor for the development of Hib disease during surveillance of Hib vaccine failures following the introduction of the vaccine into the UK immunization standard [2]. Since then, a comparatively small number of studies have assessed the safety, immunogenicity, efficacy and duration of immune responses in preterm infants compared with term infants for routinely recommended childhood immunizations [3–7]. In some of these studies, preterm infants have been shown to demonstrate a variable immune response to protein-based antigens. However, data on the newer conjugate vaccines are limited [8]. Data from studies of the immune responses of premature infants to routine immunization are limited both by sample size and the relatively small number of studies that have been performed in preterm infants.

Various theories have been proposed for the reduced immune response in preterm infants, including relative immaturity of the immune system. The use of pre- and postnatal steroids and reduced muscle mass may also contribute to a reduced response for some vaccines [4]. Whatever the reasons, these children represent a population at greater risk of infection than their term infant counterparts and their response to immunization is therefore of particular concern.

Most studies of vaccine responses in premature infants have been limited to immunogenicity studies. A small number have addressed antibody functionality by assessing avidity of antibody, and even fewer have assessed vaccine efficacy in premature infants. Some studies have also recorded adverse events, including severe events, such as apnea, in preterm infants.

In the present report, recent studies of immune responses to certain vaccines in premature infants are reviewed and the findings of representative studies reviewed are summarized.

Immunogenicity of vaccines in preterm infants: short-term antibody responses

Haemophilus influenzae vaccines

Studies of vaccines against Hib conducted in premature and extremely premature infants have consistently shown lower anti-PRP antibody

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concentrations than in term infants [5–7]. In a study conducted by Dinan in 1998, antipolyribosylribitol phosphate (PRP) responses were compared between preterm and term infants who received the diphtheria–tetanus–whole-cell pertussis vaccine (DTPw) and a conjugate Hib vaccine at 2, 4 and 6 months of age [8]. Of a total of 81 premature infants (<37 weeks), 28% had lower than protective levels (<0.15 µg/ml) of anti-PRP at 8 months of age compared with 13% of a total of 133 term infants (x^2: 8.23, p = 0.0046) (TABLE 1). There was no apparent difference in the degree of prematurity and the anti-PRP responses.

The differences in the mean anti-PRP immunoglobulin (Ig)G antibody concentrations between the term and preterm infants at 2, 4, 6 and 8 months are shown in FIGURE 1. The effect of gestational age on the outcome of anti-PRP IgG antibody concentrations was statistically significant at birth ages of 2 and 4 months (p < 0.0001 and p = 0.0008, respectively) but was not significant at birth ages of 6 and 8 months (p = 0.940 and p = 0.187, respectively) [8].

Slack and colleagues confirmed the results of earlier studies by examining Hib PRP and meningococcal serotype C immune responses in term and preterm infants immunized at 2, 3 and 4 months of age with a combined diphtheria–tetanus–acellular pertussis-Hib (DTPa–Hib) conjugate vaccine [9].

The geometric mean concentration (GMC) for Hib PRP was very low, as was the proportion of infants achieving IgG anti-PRP concentrations of greater than or equal to 0.15 or 1.0 µg/mL as detailed in TABLE 2. There was no association between the Hib anti-PRP IgG response and gestational age or weight at birth. However the IgG anti-PRP concentration was strongly associated with age at the third immunization (p < 0.001), with a 22% increase (95% confidence intervals [CI]: 11–34%) per week of age [9]. Interestingly, the Hib IgG anti-PRP concentrations appeared to be 88% higher in infants who did not receive antenatal steroids compared with those who did (0.44 and 0.24 respectively; p = 0.056), although after adjusting for age at third immunization this difference was reduced to 51% (p = 0.20).

Table 1. Degree of prematurity and percentage of infants with lower than protective levels of antibody to Hib PRP at 8 months after routine immunization at 2, 4 and 6 months of age with Hib conjugate vaccine.

Degree of prematurity	Percentage of infants with lower than protective concentrations of anti-PRP IgG antibody
<28 weeks (n = 21)	24
28–32 weeks (n = 28)	36
>32 weeks (n = 32)	25

Hib: *Haemophilus influenzae* type b; Ig: Immunoglobulin; PRP: Polyribosylribitol phosphate (summarized from Dinan *et al.* [8]).

A study by Boros and colleagues in 2002 investigated IgG anti-Hib PRP responses in premature infants following a three-dose primary schedule in term and preterm infants (TABLE 3) [10]. These results indicated that preterm infants have a significant reduction in antibody responses to PRP, and therefore possibly decreased long-term protection against Hib disease, compared with term infants.

Acellular pertussis vaccines

In 1999, Schloesser and colleagues reported investigations of the immune response of premature infants to a two-component Pa vaccine given at 2 months of age, then at 2–4 monthly intervals for a total of three doses [11]. This study showed a significant reduction in antibody responses to pertussis toxin (PT) and filamentous hemagglutinin (FHA) pertussis antigens in preterm infants (25–35 weeks gestation) as shown in TABLE 4.

In contrast to diphtheria and tetanus immunization, in which a protective antibody level has been determined, protective levels for pertussis immunization are unknown. In addition, antibody responses do not correlate well with efficacy of immunization for pertussis. The lower antibody response seen in preterm infants is of potential concern when considering long-term protection.

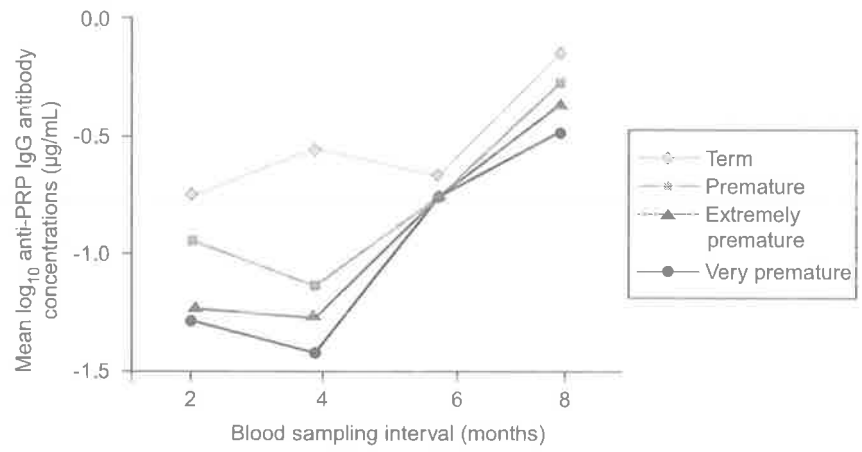

Figure 1. Mean anti-PRP IgG antibody concentrations in relation to Hib conjugate vaccine administration (expressed as $\log_{10}$) for term and preterm infant groups at birth ages of 2, 4, 6 and 8 months [8].
Ig: Immunoglobulin; PRP: Polyribosylribitol phosphate.

Poliovirus vaccines

In a study reported by Linder and colleagues in 2000, antibody responses to polio vaccine were compared in 35 term and 52 preterm infants (30–35 weeks) following routine immunization with inactivated polio vaccine (IPV) at 2 and 4 months and oral polio vaccine (OPV) at 4 and 6 months [12]. This study did not identify any significant difference in

Table 2. Hib antibody responses in premature and term infants 1 month after the third immunization with Hib conjugate vaccine.

	PRP GMC µg/mL (95% CI)	IgG > 0.15 µg/mL	IgG > 1.0 µg/mL
Preterm (n = 105)	0.27 (0.21–0.35)	55%	21%
Term (n = 54)	0.81 (0.52–1.25)	80%	46%
	p < 0.001	p < 0.003	p < 0.0012

CI: Confidence interval; Hib: *Hemophilus influenzae* type b; GMC: Geometric mean concentration; Ig: Immunoglobulin; PRP: Polyribosylribitol phosphate. (summarized from Slack *et al.* [9]).

antibody titers and GMTs for poliovirus serotypes 1, 2 and 3 between the preterm and term study groups at 7 months, although there was a significant difference in IgG antibody titers between term and preterm infants for poliovirus Type 3 at 3 months of age.

A study by Kirmani and colleagues described similar results for immunization of premature infants with poliovirus vaccines [13].

Hepatitis B vaccines

In the same study that described responses to poliovirus immunization, preterm and term antibody responses to hepatitis B were examined [12,14]. There was a significant difference between the percentage of preterm and term infants with antibody titers of 1:10 or more at 7 months of age.

Kim and colleagues examined hepatitis B IgG antibody responses in extremely premature and premature infants (23–36 weeks gestation) [15]. Hepatitis B vaccine was administered at birth and at 1 and 6 months of age. The study was conducted in infants of mothers who were hepatitis B surface antigen (HBsAg) negative. Of 87 subjects enrolled, 90% demonstrated seroprotection (anti-HBs > 10mIU/mL) after three doses of vaccine. The geometric mean antibody titer (GMT) to HBsAg for infants who seroconverted was 200 mIU/mL, compared with the response seen in a study by West and colleagues in which term infants had a mean GMT of 647 mIU/mL [16].

Table 3. Hib PRP GMCs and percentage of infants with a GMC > 1µg/mL by gestational age.

Gestation	GMC µg/mL	% > 1 µg/mL
< 28 weeks (n = 11)	1.76[§]	50.0
28–32 weeks (n – 13)	1.59[§]	62.5
33–37 weeks (n = 17)	6.74	87.5
> 37 weeks (n 54)	6.75	98.0

[§]: Significantly lower than term infants.
Hib: *Hemophilus influenzae* type b; GMC: Geometric mean concentration; PRP: Polyribosylribitol phosphate (summarized from Boros [10]).

Table 4. GMT for PT and FHA antibodies after the third immunization with a two component acellular pertussis vaccine.

Gestation	PT GMT (95% CI)	FHA GMT (95% CI)
Preterm infants (25–35 weeks)	64.2 (51.4–80.1)	50.9 (42.3–61.3)
Term	98.9 (p < 0.003) (81.6–120.0)	86.0 (p < 0.0001) (72.5–102.1)

CI: Confidence interval; GMT: Geometric mean titer; FHA: Filamentous hemagglutinin; PT: Pertussis toxin (summarized from Schloesser *et al.* [11]).

Meningococcal conjugate C vaccine

Meningococcal conjugate C (MCC) vaccine was recently included in the Australian Standard Vaccination Schedule following its successful introduction in the UK. Slack and colleagues compared the antibody responses of term and preterm infants with Hib and MCC vaccines in the UK [9]. A total of 105 infants born at less than 32 weeks gestation had Hib IgG GMCs and MCC serum bactericidal antibody (SBA) GMTs determined 1 month after the third immunization (immunizations given at 2, 3 and 4 months) [9]. In comparison with many of the previous antigens discussed, both preterm and term infants showed a similarly good response to MCC immunization (TABLE 6).

SBA GMTs to MCC and the proportions of preterm infants achieving an SBA titers of eight or 128 or more were similar to those achieved by term infants (TABLE 6). A total of 99% of premature infants showed a four-fold or greater rise in SBA titer following immunization with three doses of MCC. Of note, the administration of antenatal steroids did not adversely affect the IgG antibody response.

Pneumococcal vaccines

The seminal study of heptavalent pneumococcal conjugate vaccine conducted by Shinefield and Black in the Kaiser Permanente centers in the USA investigated the immunogenicity of pneumococcal vaccine in 38,000 infants, of whom 4340 were born at less than 38 weeks gestation [17]. Similar immune responses to all seven pneumococcal serotypes were found for full term and preterm infants.

Immunogenicity of vaccines in preterm infants: longer-term antibody responses

Some investigators have studied longer-term antibody responses in premature infants who have received a variety of vaccines.

A study of 41 preterm infants and 54 term infants conducted by Boros and colleagues demonstrated reduction in antibody responses in preterm infants compared with term following DTPa and Hib booster doses given at 18 months of age as part of the routine immunization schedule [10]. IgG antibody concentrations for pertussis antigens (PT, FHA, pertactin), diphtheria, tetanus and Hib PRP were examined. The differences between the preterm and term groups are summarized in TABLE 7 for each antigen. In general, lower GMCs were found with earlier gestation.

Table 5. GMTs for hepatitis B surface IgG antibody and percentage of infants with antibody titers >1:10 at 7 months of age after hepatitis B vaccine administration.

Gestation	HBs GMT at 7 months	% titer > 1:10
Preterm (30–35 weeks)	420	79 (<0.05)
Term	653	94

HB: Hepatitis B; GMT: Geometric mean titer (summarized from Linder *et al.* [12]).

A long-term follow-up study of preterm infants was conducted by Esposito and colleagues [18]. Antibody titers against pertussis antigens were evaluated at 5–6 years of age in children who had been preterm and were compared with responses for children who had been born at term. Children enrolled in the study were immunized with DTPa–hepatitis B vaccine (HBV) at 3, 5 and 11 months. The results of this study revealed that long-term immune responses induced by primary pertussis immunization in preterm infants (especially ≤ 31 weeks) were qualitatively and quantitatively lower than those observed in term infants [18].

The study by Kirmani and colleagues of infants born at less than 29 weeks gestation demonstrated evidence of reduced long-term antibody responses to many of the recommended vaccines, as shown in TABLE 8 [13].

Antibody functionality: studies of antibody avidity to vaccine antigens in premature infants

The evidence presented above suggests that preterm infants have reduced antibody responses to many of the routine immunizations. Less is known about *in vitro* antibody functionality, as represented by antibody avidity, in preterm infants. Antibody avidity refers to the ability of an antibody mixture (for example, antibody in serum) to bind to the antigen in question, and is important in enabling antibody function, such as opsonization or microbial killing. Antibody avidity to Hib PRP was examined in a study by Dinan and colleagues and was found to be reduced compared with avidity in term infants [8]. The study of Kirmani and colleagues reported in 2002 compared antibody avidity with Hib PRP and diphtheria toxoid at 7 years of age in extremely premature and term infants and found avidity to be similar in both groups. However the sample size was small in this study [13].

Table 6. Meningococcal serogroup C serum bactericidal antibody titers in premature and term infants after meningococcal C conjugate vaccine administration.

Antibody response	Preterm infants (n=105)	Term infants (n=54)	p-value
MCC SBA GMT	398 (95% CI: 298–532)	380 (275–526)	0.44
SBA > 8	104 (99)	53 (98)	1.00

GMT: Geometric mean titer; MCC: Meningococcal serogroup C; SBA: Serum bactericidal antibody (summarized from Slack *et al.* [9]).

Table 7. Difference between term and preterm antibody concentrations for six vaccine antigens at 19 months of age.

Antigen	Statistical significance (student's t-test)
PT	p = 0.001
FHA	p = 0.007
PRN	p = 0.036
Diphtheria	p = 0.673
Tetanus	p = 0.014
Hib PRP	p = 0.186

FHA: Filamentous hemagglutinin; Hib: *Haemophilus influenzae* type b; PRN: Pertactin; PRP: Polyribosylribitol phosphate; PT: Pertussis toxin (summarized from Boros [10]).

Efficacy studies of vaccines in preterm infants

By their very nature, it is extremely difficult to perform efficacy studies of vaccines in premature infants, as the study numbers required are very large. Results from the Kaiser Permanente study demonstrated adequate protection in the 4340 preterm infants immunized with 7-valent pneumococcal vaccine (Prevnar®, Wyeth) [17]. No child immunized with Prevnar developed invasive pneumococcal disease due to any of the vaccine serotypes compared with nine cases in the controls [17].

Table 8. Antibody titers to seven vaccine antigens at 6–7 years of age (<29 weeks and <1000 g).

Level of antibody	Preterm	Term	p-value
Diphtheria toxoid GMT > 0.1 IU/mL	0.37 (81%)	1.07 (100%)	0.009
Tetanus toxoid GMT > 0.01	1.99 (100%)	4.22 (100%)	0.04
Hib-PRP µg/mL > 0.15 µg/mL > 1.0 µg/mL	1.41 (100%) (62%)	3.21 (100%) (75%)	 0.03 0.45
Polio serotype 1 GMT > 1:8	215 (100%)	181 (100%)	0.54
Polio serotype 2 GMT > 1:8	128 (100%)	206 (100%)	0.09
Polio serotype 3 GMT > 1:8	24 (75%)	59 (100%)	0.06
HBsAb GMT > 10 mIU/mL	186 (86%)	120 (69%)	0.62

GMT: Geometric mean titer; HBsAg: Hepatitis B surface antigen; Hib: *Haemophilus influenzae* type b; PRP: Polyribosylribitol phosphate (summarized from Kirmani *et al.* [13]).

Conclusions

Recent evidence suggests that preterm infants have significant impairment in IgG antibody responses to a number of routine immunization antigens. Most studies have been performed with small numbers of infants. However the results are consistent across studies. Importantly, IgG antibody responses to Hib, pertussis and HBV are reduced in this at-risk population. The evidence suggests that these reduced antibody responses persist throughout childhood. Antibody avidity has been shown to be reduced in preterm infants, although it appears for some antigens that avidity levels approach those seen in term infants by later childhood. Efficacy studies in this at-risk population are difficult to perform due to the large number of subjects required and the costs involved.

The current recommendations in Australia and internationally are to immunize preterm infants at their appropriate chronological age using the routine schedule and to include 7-valent pneumococcal immunization [18]. The 8th edition of the Australian Immunization Handbook includes preterm infants of less than 28 weeks as a high-risk group for invasive pneumococcal disease and therefore funding is provided for infants in this group to receive pneumococcal immunization [20]. The 7-valent pneumococcal conjugate vaccine should be offered at 2, 4 and 6 months of age, with a fourth dose at 12 months of age and a 23-valent pneumococcal polysaccharide vaccine booster at 4–5 years of age. Extremely premature infants, of less than 28 weeks, who receive PedvaxHib® (Merck) should receive an additional dose at 6 months of age. As preterm infants do not respond as well to HBV as term infants, those less than 32 weeks gestation either require serology to be performed at 7 months of age to assess immunity (with the possibility of an additional booster if antibody concentrations are low) or a four dose schedule at 2, 4, 6 and 12 months (only permissible if the mother is known to be hepatitis B seronegative). Further studies to assess the functionality of antibody responses as a measure of potential long-term protection in premature infants are required. Similar studies in small for gestational age infants would also be of benefit.

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Reactogenicity and immunogenicity profile of a two-dose combined hepatitis A and B vaccine in 1–11-year-old children

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Abstract

This study was conducted to compare the reactogenicity, immunogenicity and safety of a combined two-dose (0, 6 months) hepatitis A and B vaccine (720 EL U HAV, 20 mcg HBsAg) with the established three-dose (0, 1 and 6 months) hepatitis A and B vaccine (360 EL U HAV, 10 mcg HBsAg). A total of 511 children aged 1–11 years who had not previously received a hepatitis A or B vaccine were enrolled in the study. Both vaccines were well tolerated, and were shown to be safe and immunogenic. The analysis, stratified according to two age groups (1–5 year and 6–11-year-old children) demonstrated that the reactogenicity profile of the two-dose schedule was at least as good as that of the established schedule. Both vaccines and schedules provided at least 98% seroprotection against hepatitis B and 100% seroconversion against hepatitis A, 1 month after the end of the vaccination course (Month 7).

Keywords: Hepatitis A and B; Combined vaccine; Children

1. Introduction

Hepatitis A and B represent the most frequent forms of viral infections of the liver, causing serious morbidity and mortality throughout the world. Approximately 1.4 million cases of hepatitis A are reported every year and it is estimated that there are 380 million chronic carriers of hepatitis B worldwide who have an increased risk of developing cirrhosis or primary hepatocellular carcinoma [1,2].

Vaccination has been recognized by world health authorities as the most efficient form of prophylaxis, providing long-term protection against clinical disease and infection [3,4]. Monovalent vaccines against both hepatitis A and B

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have been available for some time and have proven to be effective and safe [5–10]. However, in spite of both viruses being very different, as well as their modes of transmission [11], the incidence and importance of the disease manifestations suggest that the use of combined hepatitis A and B vaccination, would provide increased convenience and acceptance, and reduced costs of administration [12]. GlaxoSmithKline Biologicals has developed a combined hepatitis A and B vaccine licensed for both pediatric or adolescent and adult populations, according to a 0, 1, 6 month schedule. Experience with this vaccine has shown excellent immunogenicity, coupled with a good tolerability and safety profile [13–15].

The possibility of providing a two-dose schedule for the combined vaccine offers further benefits in terms of compliance and patient acceptability. A two-dose schedule, using either a 0, 6 or 0, 12 month regimen has been shown to be effective and safe in children 1–15 years of age [16–19].

The primary objective of this study was to show non-inferiority with regards to reactogenicity of the combined two-dose hepatitis A and B vaccine with double antigen content at 0 and 6 months with the established three-dose combined hepatitis A and B vaccine administered at 0, 1, and 6 months in subjects aged 1–11 years, stratified into two age groups, from 1 to 5 and 6 to 11 years, respectively. The primary endpoint was the overall number of subjects reporting at least one grade 3 solicited symptom on the day of vaccination and during the 4 day follow-up period after each vaccine dose. The secondary objective was to assess the safety and immunogenicity of the combined two-dose hepatitis A and B vaccine with double antigen content at 0 and 6 months compared with the established three-dose combined hepatitis A and B vaccine.

2. Materials and methods

2.1. Subjects

This was an international, multi-centre study in which children were enrolled from 12 centres; 2 in Australia, 3 in Sweden, 6 in Spain and 1 in Belgium. Children were recruited from the community. No incentive payments were provided for participation in the study. Subjects were to be free of obvious health problems as established by medical history and clinical examination. They were also required not to have been previously vaccinated against hepatitis A and B and to have no history or evidence of hepatitis A or B disease, confirmed by serology at screening. Although very unlikely in this age group, female subjects who were at risk of becoming pregnant were to take precautions to avoid pregnancy. Confirmed or suspected disorders of the immune system, major congenital defects (including immunodeficiency), serious chronic illness, acute disease or a body temperature $\geq$37.5 °C at the time of enrolment excluded subjects from entry into the study. None of the subjects had received investigational or non-registered drugs or vaccines within 30 days of vaccination and none had received immune modifying drugs within 6 months of entry. The use of additional investigational drugs and vaccines was prohibited during the course of study.

This study was conducted in accordance with Good Clinical Practice guidelines and with the amended Declaration of Helsinki in force at the time of study start. Independent Ethics Committees gave approval prior to study start in each centre.

2.2. Vaccines

Both vaccines used in the study were developed and manufactured by GlaxoSmithKline Biologicals, Rixensart, Belgium. Two-dose combined hepatitis A and B vaccine (registered as Ambirix in Europe only and as Twinrix Adult in the rest of the world) contained 720 EL.U of inactivated hepatitis A virus, 20 μg of recombinant hepatitis B antigen and 0.45 mg of aluminium as salt in a 1 ml dose (HepA/B 720/20). Established three-dose combined hepatitis A and B vaccine, Twinrix Junior (HepA/B 360/10), contained 360 EL U of inactivated hepatitis A virus, 10 μg of recombinant hepatitis B antigen and 0.225 mg of aluminium as salt in a 0.5 ml dose.

2.3. Study design

Subjects were randomized on an open label basis to one of two parallel treatment groups. The randomisation was made using a standard Statistical Analysis System (SAS®) program. The randomisation was stratified according to the age group (1–5 year olds versus 6–11 year olds) and the centre. A randomisation number identified uniquely the vaccine doses administered to the same subject. One group of subjects was vaccinated with the three-dose schedule (360 EL U HAV, 10 mcg HBsAg) according to a 0, 1 and 6 month schedule and the other group was vaccinated with the two-dose schedule (720 EL U HAV, 20 mcg HBsAg) according to a 0 and 6 month schedule. Vaccines were administered intramuscularly in the left deltoid region.

2.4. Reactogenicity and safety

Solicited local (injection site pain, redness and swelling) and general symptoms (drowsiness, irritability/fussiness, loss of appetite and fever in subjects aged 5 years or less; fatigue, gastro-intestinal symptoms, headache and fever in subjects aged 6–11 years) were recorded on diary cards by the parent or guardian of each subject for 4 days after each vaccination. Each reaction was scored as one of the following: grade 1 (easily tolerated), grade 2 (interfered with daily activities) and grade 3 (prevented daily normal activities), also documenting duration of the symptoms. Redness and swelling were measured and scored as grade 1 (1–5 mm for subjects aged 5 years or less and 1–10 mm for subjects aged 6–11 years), grade 2 (5–25 mm for subjects aged 5 years or less and

10–50 mm for subjects aged 6–11 years) or grade 3 (>25 mm for subjects aged 5 years or less and >50 mm for subjects aged 6–11 years). Axillary or oral body temperature was scored as grade 1 (37.5–38.5 °C), grade 2 (38.6–39.5 °C) or grade 3 (>39.5 °C). When temperature was recorded by the rectal route, the intensity scale cut-offs were taken as 0.5 °C more than the cut-offs given above. All local signs and symptoms were considered to be related to vaccination. Unsolicited signs and symptoms were recorded during 30 days after each vaccine dose by the investigator. Serious adverse events were reported throughout the study period. The relationship of all solicited general symptoms and unsolicited adverse events (serious or non-serious) to vaccination was assessed by the investigator as follows: 'not causally related' or 'reasonable possibility that the vaccine contributed to the adverse event'.

2.5. Serology

All subjects were screened for the presence of anti-HAV (Enzygnost®, DADE Behring), anti-HBs (AUSAB EIA®, Abbott), anti-HBc (Axsym Core, Abbott) antibodies and HBsAg (Axsym HBsAg, Abbott) prior to vaccination. At Month 7, 1 month after the end of the vaccination schedule, a blood sample (3–5 ml) was taken to assay anti-HAV and anti-HBs antibodies. Seropositivity rate was defined as the percentage of subjects with anti-HAV antibody titres ≥15 mIU/ml and a subject was said to be seroprotected against hepatitis B infection if anti-HBs antibody titres were ≥10 mIU/ml. GMT calculations were performed by taking the anti-log of the mean of the log titre transformations.

2.6. Statistical analysis

Two hundred eligible subjects per group were needed to conclude non-inferiority with at least 80% power, assuming that the formulations were equally reactogenic. Non-inferiority was assessed by computing an exact one-sided 95% confidence interval (CI) ($\alpha = 2.5\%$) on the difference in percentage, the non-inferiority limit being set at 10%. Allowing for 10% of subjects who dropped-out or were not eligible for analysis, 440 subjects (220 per group) were planned to be enrolled.

Demographic characteristics (age, gender, and race) were analyzed descriptively. Percentages of subjects presenting symptoms (solicited/unsolicited, local/general) were calculated. The incidence, intensity and relationship of individual solicited and unsolicited symptoms and serious adverse events occurring during the study period were analyzed descriptively. The reactogenicity analysis was stratified according to two age groups (1–5 and 6–11-year-old subjects). Anti-HAV and anti-HBs antibody seropositivity rates, anti-HBs antibody seroprotection rates, and GMTs with their 95% CI were calculated. The According to Protocol (ATP) cohort for analysis of reactogenicity included all subjects who had received at least one dose of vaccine according to their random assignment, with sufficient data to perform an analysis and who had not received a vaccine not specified or forbidden in the protocol. The ATP cohort for analysis of immunogenicity included all eligible subjects (i.e. those meeting all eligibility criteria complying with the procedures defined in the protocol, for whom data concerning immunogenicity endpoint measures were available).

3. Results

In total, 511 subjects of either gender, aged between 1 and 11 years at the time of the first vaccination were recruited into the study after written informed consent was obtained from their parent or guardian. The study was conducted between September 2001 and July 2002, with all subjects enrolled over a 2-month period.

3.1. Demographic data

Of the 511 subjects enrolled (255 in the HepA/B 720/20 group and 256 in the HepA/B 360/10 group), 498 completed the study and 13 dropped out (six protocol violations, six randomization failures and one case of no vaccine administration). None of the drop-outs was linked to an adverse event. The number of subjects included in the ATP cohort for reactogenicity and immunogenicity were, respectively, equal to 495 (248 in the HepA/B 720/20 group and 247 in the HepA/B 360/10 group, as three subjects did not complete symptom sheets) and 395 (204 in the HepA/B 720/20 group and 191 in the HepA/B 360/10 group). Ninety-three subjects were eliminated from the ATP cohort for immunogenicity due to initially seropositive (21) or unknown antibody status (72); two protocol violations; six non-compliance with vaccination schedule and two non-compliance with blood sampling schedule.

Overall, in the total cohort, mean age was 6.0 ± 3.1 years (mean ± S.D.) and 55% of the subjects were male. The median age was 6 years with a minimum age of 1 year and a maximum age of 12 years (three subjects were out of the protocol-specified age range of 1–11 years by only a few days). In the 1–5 year group, mean age was 3.1 ± 1.3 years, the median age was 3 years and 56.5% of the subjects were male. In the 6–11 year group, mean age was 8.7 ± 1.6 years, the median age was 9 years and 53.8% of the subjects were male. The demographic profile of the two vaccine groups was comparable with respect to mean age, gender and racial distribution in both age groups and in all three cohorts.

3.2. Reactogenicity and safety

During the 4-day follow up period after all vaccinations, of the 248 children who received the HepA/B 720/20 vaccine, 12 (4.8%) reported at least one grade 3 local and/or general symptom versus 16 (6.5%) out of 247 subjects in the HepA/B 360/10 vaccine group. The −1.7% (−7.07; 3.60 CI) percentage difference between the two groups was below

Table 1
Number and percentage of subjects with solicited local symptoms (ATP cohort)

Solicited local symptom	Total ATP cohort (N=495)				p-Value[a]	1–5 year old (N=233)				p-Value[a]	6–11 year old (N=262)				p-Value[a]
	HepA/B 720/20 (N=248)		HepA/B 360/10 (N=247)			HepA/B 720/20 (N=111)		HepA/B 360/10 (N=122)			HepA/B 720/20 (N=137)		HepA/B 360/10 (N=125)		
	n	Percentage	n	Percentage		n	Percentage	n	Percentage		n	Percentage	n	Percentage	
Pain															
All	134	54.0 (47.6–60.4)	128	51.8 (45.4–58.2)	0.6529	53	47.7 (38.2–57.4)	56	45.9 (36.8–55.2)	0.7940	81	59.1 (50.4–67.4)	72	57.6 (48.4–66.4)	0.9002
G 3	5	2.0 (0.7–4.6)	3	1.2 (0.3–3.5)	0.7243	2	1.8 (0.2–6.4)	1	0.8 (0.0–4.5)	0.6063	3	2.2 (0.5–6.3)	2	1.6 (0.2–5.7)	1.0000
Redness															
All	62	25.0 (19.7–30.9)	76	30.8 (25.1–36.9)	0.1615	30	27.0 (19.0–36.3)	45	36.9 (28.3–46.1)	0.1234	32	23.4 (16.6–31.3)	31	24.8 (17.5–33.3)	0.8850
G 3	1	0.4 (0.0–2.2)	3	1.2 (0.3–3.5)	0.3725	1	0.9 (0.0–4.9)	2	1.6 (0.2–5.8)	1.0000	0	0.0 (0.0–2.7)	1	0.8 (0.0–4.4)	0.4771
Swelling															
All	39	15.7 (11.4–20.9)	52	21.1 (16.1–26.7)	0.1329	26	23.4 (15.9–32.4)	29	23.8 (16.5–32.3)	1.0000	13	9.5 (5.1–15.7)	23	18.4 (12.0–26.3)	0.0475
G 3	1	0.4 (0.0–2.2)	4	1.6 (0.4–4.1)	0.2159	1	0.9 (0.0–4.9)	2	1.6 (0.2–5.8)	1.0000	0	0.0 (0.0–2.7)	2	1.6 (0.2–5.7)	0.2267

N: total number of subjects with at least one documented dose, n: number of subjects reporting at least one type of symptom during the 4-day follow-up period, G3: grade 3; pain grade 3: spontaneously painful, redness/swelling grade 3: with greatest surface diameter >25 mm (for children aged 1–5 years) or >50 mm (for children aged 6–11 years), grade 3: prevented normal activity.

[a] Fisher's 2-sided test.

the pre-defined 10% limit for non-inferiority of the HepA/B 720/20 vaccine to be demonstrated.

In children aged 1–5 years, the overall incidence of solicited local symptoms was similar in both groups. Subjects in both groups reported pain at the injection site most frequently: 53 of 111 subjects (47.7%) in the HepA/B 720/20 group and 56 of 122 subjects (45.9%) in the HepA/B 360/10 group. Only nine subjects (four in the HepA/B 720/20 group and five in the HepA/B 360/10 group) reported local symptoms of grade 3 intensity, which all resolved without sequelae. In children aged 6–11 years, the overall incidence of solicited local symptoms was similar in both groups, except for swelling which tended to be higher at 18.4% (23 of 125 subjects) in the HepA/B 360/10 group than in the HepA/B 720/20 group at 9.5% (13 of 137 subjects). Subjects in both groups reported pain at the injection site most frequently: 81 of 137 subjects (59.1%) in the HepA/B 720/20 group and 72 of 125 subjects (57.6%) in the HepA/B 360/10 group. Only eight subjects (three in the HepA/B 720/20 group and five in the HepA/B 360/10 group) reported local symptoms of grade 3 intensity, which all resolved without sequelae (Table 1).

In children aged 1–5 years, subjects in both groups reported irritability/fussiness most frequently: 35 of 111 subjects (31.5%) in the HepA/B 720/20 group and 52 of 122 subjects (42.6%) in the HepA/B 360/10 group. The majority of the general symptoms reported were considered by the investigator to be related to vaccination. Only seven subjects (two in the HepA/B 720/20 group and five in the HepA/B 360/10 group) reported symptoms of grade 3 intensity, which were considered by the investigator to be related to vaccination. All symptoms resolved without sequelae.

In children aged 6–11 years, subjects in both groups reported fatigue and headache most frequently: 29 of 137 subjects (21.2%) and 25 of 137 subjects (18.2%) respectively, in the HepA/B 720/20 group and 36 of 125 (28.8%) and 40 of 125 (32%), respectively, in the HepA/B 360/10 group. The majority of the general symptoms were considered by the investigator to be related to vaccination. Only three subjects (in the HepA/B 360/10 group) reported symptoms of grade 3 intensity, which were considered by the investigator to be related to vaccination. All symptoms resolved without sequelae (Table 2).

A total of 82 subjects (39 in the HepA/B 720/20 group and 43 in the HepA/B 360/10 group) reported at least one unsolicited symptom, which was considered by the investigator to be related to vaccination, during the 31-day follow-up period after vaccination. A total of 75 subjects (33 in the HepA/B 720/20 group and 42 in the HepA/B 360/10 group) reported unsolicited symptoms of intensity grade 3 during the 31-day follow-up period after vaccination and seven of these unsolicited symptoms (3 in the HepA/B 720/20 group and four in the HepA/B 360/10 group) were considered by the investigator to be related to vaccination (data not shown). 98.6% of all unsolicited symptoms resolved during the 31-day follow-up period. During the entire study period, only one subject (in the HepA/B 360/10 group) reported an SAE considered by the investigator to be related to vaccination. It consisted of vomiting, fever with symptoms of rhinorrhea and cough four days after receiving the first vaccine dose. The subject was hospitalized and diagnosed with viral upper respiratory tract infection and exacerbation of eczema. After recovery, the subject received the second and the third vaccine doses without any further SAE.

All results obtained in the ATP cohort for reactogenicity were confirmed in the total cohort.

3.3. Immunogenicity

At month 7 (1 month post-vaccination course), 100% of subjects were seropositive for anti-HAV antibodies and more than 98% of subjects had seroprotective levels of anti-HBs antibodies. High GMTs were measured in both groups (>8400 mIU/ml for anti-HAV and >7800 mIU/ml for

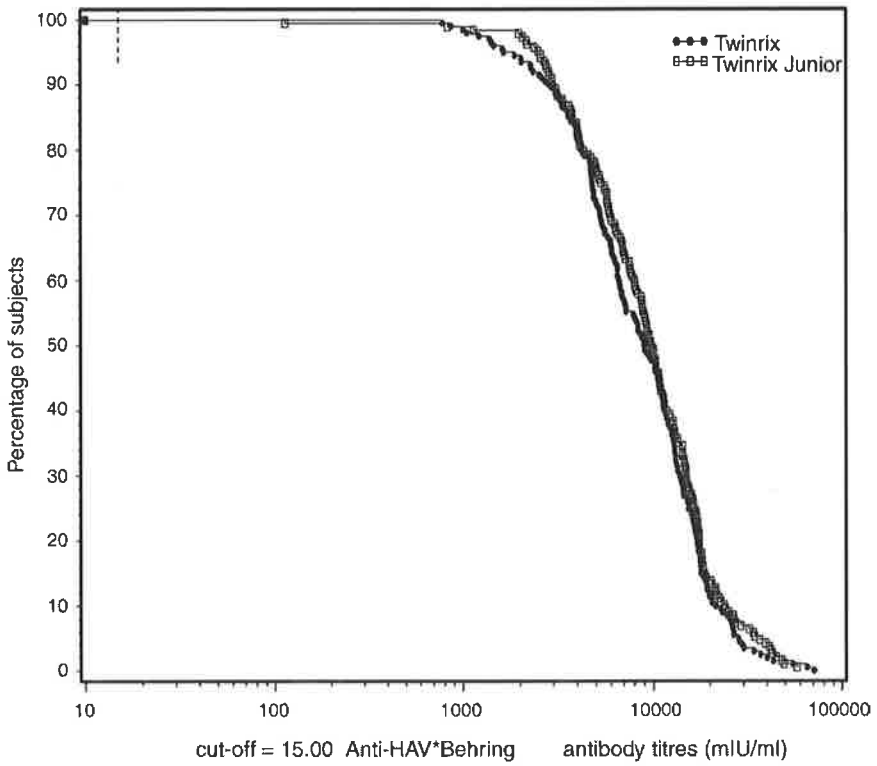

Fig. 1. Reverse cumulative distribution curves (RCC) for anti-HAV antibodies post-vaccination (Month 7) in HepA/B 360/10 ("Twinrix Junior") vs. Hep A/B 720/20 ("Twinrix") groups.

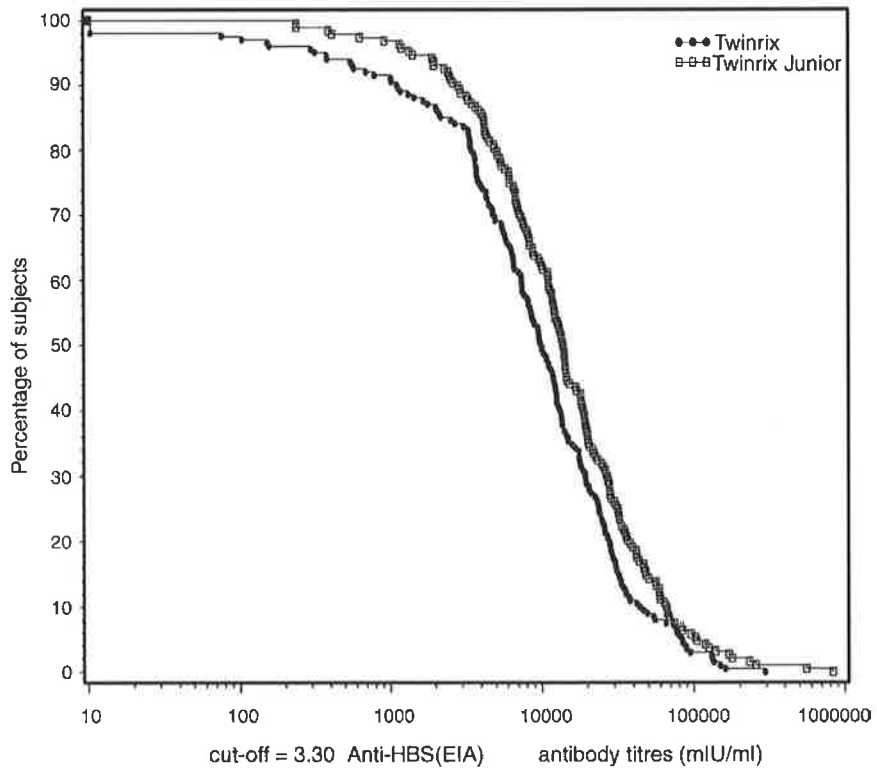

Fig. 2. Reverse cumulative distribution curves (RCC) anti-HBs antibodies post-vaccination (Month 7) in HepA/B 360/10 ("Twinrix Junior") vs. Hep A/B 720/20 ("Twinrix") groups.

Table 2
Number and percentage of subjects with solicited general symptoms (ATP cohort)

1–5 year old		HepA/B 720/20 ($N=111$)		HepA/B 360/10 ($N=122$)		p-Value[a]
		n	Percentage	n	Percentage	
Drowsiness	All	24	21.6 (14.4–30.4)	39	32.0 (23.8–41.0)	0.0790
	Related/G3	1	0.9 (0.0–4.9)	3	2.5 (0.5–7.0)	0.6234
Irritability/fussiness	All	35	31.5 (23.0–41.0)	52	42.6 (33.7–51.9)	0.1035
	Related/G3	0	0.0 (0.0–3.3)	1	0.8 (0.0–4.5)	1.0000
Loss of appetite	All	20	18.0 (11.4–26.4)	37	30.3 (22.3–39.3)	0.0331
	Related/G3	1	0.9 (0.0–4.9)	0	0.0 (0.0–3.0)	0.4764
Fever	All	13	11.7 (6.4–19.2)	19	15.6 (9.6–23.2)	0.4487
	Related/G3	0	0.0 (0.0–3.3)	1	0.8 (0.0–4.5)	1.0000
6–11 year-old		$N=137$		$N=125$		
		n	Percentage	n	Percentage	
Fatigue	All	29	21.2 (14.7–29.0)	36	28.8 (21.1–37.6)	0.1973
	Related/G3	0	0.0 (0.0–2.7)	1	0.8 (0.0–4.4)	0.4771
Gastro-intestinal	All	20	14.6 (9.2–21.6)	26	20.8 (14.1–29.0)	0.1976
	Related/G3	0	0.0 (0.0–2.7)	1	0.8 (0.0–4.4)	0.4771
Headache	All	25	18.2 (12.2–25.7)	40	32.0 (23.9–40.9)	0.0145
	Related/G3	0	0.0 (0.0–2.7)	1	0.8 (0.0–4.4)	0.4771
Fever	All	8	5.8 (2.6–11.2)	16	12.8 (7.5–20.0)	0.0564
	Related/G3	0	0.0 (0.0–2.7)	0	0.0 (0.0–2.9)	–

N: total number of subjects with at least one documented dose, n: number of subjects reporting at least one type of symptom during the 4-day follow-up period, related: symptoms considered by the investigator to be related to vaccination, grade 3: prevented normal activity, fever: oral/axillary temperature $\geq 37.5\,^{\circ}C$ or rectal temperature $\geq 38\,^{\circ}C$, fever grade 3: oral/axillary temperature $>39.5\,^{\circ}C$ or rectal temperature $>40\,^{\circ}C$, irritability grade 3: crying that could not be comforted/prevented normal activity, loss of appetite grade 3: not eating at all.

[a] Fisher's 2-sided test.

Table 3
Post-vaccination (Month 7) anti-HBs seroprotection rates, anti-HAV seropositivity rates and geometric mean titres (ATP cohort)

	Time	Anti-HBs antibodies		Anti-HAV antibodies	
		SP (%)	GMT (95% CI) (mIU/ml)	S+ (%)	GMT (95% CI) (mIU/ml)
HepA/B 720/20 ($N=201$)	Month 7	98.5	7894 (6131–10165)	100	8412 (7483–9457)
HepA/B 360/10 ($N=188$)	Month 7	100	13683 (11315–16548)	100	9257 (8160–10501)

N: number of subjects with serology results available, SP: seroprotection for anti-HBs antibodies (i.e. titres ≥ 10 mIU/ml), S+: seropositivity for anti-HAV antibodies (i.e. titres ≥ 15 mIU/ml), GMT: geometric mean titres, 95% CI: 95% confidence interval.

anti-HBs) (Table 3). Reverse cumulative distribution curves (RCC) of anti-HAV and anti-HBs antibody titres are presented, respectively, in Figs. 1 and 2. The results obtained in the ATP cohort for immunogenicity were confirmed in the total cohort.

4. Discussion

The results of the present study clearly demonstrate that both two-dose combined hepatitis A and B vaccine with double antigen content (HepA/B 720/20) and established three-dose schedule (HepA/B 360/10) were well tolerated and highly immunogenic in children aged 1–11 years. A good tolerability profile was documented in both age groups with both vaccines. The percentage of subjects reporting any grade 3 symptom was low in both treatment groups, not exceeding 8.5%. Moreover, the percentage of subjects reporting grade 3 fever did not exceed 1% and only one subject reported grade 3 fever considered by the investigator to be related to vaccination. Non-inferiority of the two-dose schedule versus three-dose schedule was demonstrated in terms of the percentage of subjects reporting any grade 3 (severe) solicited symptom (local and/or general). Both vaccines and schedules provided at least 98% seroprotection against hepatitis B and 100% seroconversion against hepatitis A, 1 month after the end of the vaccination course (month 7). GMTs observed at month 7 were very high in both groups for both antigens (>8400 mIU/ml for anti-HAV and >7800 mIU/ml for anti-HBs), therefore the difference observed between anti-

HBs GMT after the 3-dose (Hep A/B 360/10) versus the 2-dose schedule (Hep A/B 720/20), 13683 mIU/ml versus 7894 mIU/ml, respectively, is hardly likely to be clinically relevant. This is confirmed by anti-HBs RCCs, showing that >90% of all subjects had anti-HBs GMT $\geq$ 1000 mIU/ml at month 7.

These results confirm other recent studies wherein the immunogenicity and safety profile of a two-dose schedule (0 and 6 months) of the increased antigen content of the combined hepatitis A and B vaccine was investigated in children aged 1–11 years [14,16,19], as well as in adolescents aged 12–15 years [14,16–18].

Results from these studies indicated that this two-dose schedule could be considered an alternative for immunization of children and adolescents who are not at immediate risk of hepatitis B infection. It is particularly justified for children and adolescents in the context of school-based immunization programs. The two-dose schedule was shown to be cost-effective [20], ensuring higher coverage rates as a result of fewer injections and the avoidance of missed vaccination opportunities, a two-dose regimen offers savings in syringes, vaccine storage and cold chain, transportation, medical visits, logistics and administration costs. Considering the reduction in health care budgets, a two-dose regimen could thus provide a less costly alternative.

Acknowledgements

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Summary: Contribution and Impact

This thesis describes a body of work undertaken between 1997 and 2009 on research in vaccinology. The global and national importance of this topic demands research of the highest standards directed at answering not only how immunogenic a vaccine is but how safe it is and how well it is accepted into the community to provide the best protection for populations. Misinformation in the media about vaccines needs to be addressed by carefully designed and conducted epidemiological, clinical and community based studies.

The following provides a brief summary of the conclusions derived from the studies described in this thesis and outlines immediate and longer term impact and initiatives arising from them.

Combination Vaccines

DTPa based combination vaccines

Chapter 1 describes my research contributions to the area of combination vaccines and their application in childhood. Combination paediatric vaccines are emerging in the marketplace, bringing with them the prospect of higher coverage rates, fewer needles, greater protection against multiple diseases and more efficient products using new, more sophisticated technology. By reducing the number of injections given at each immunisation encounter, combination vaccines increase convenience of immunisations for both the vaccinees and immunisation providers. In doing so, they have the advantage of potentially higher compliance with immunisation programs and reduction of their overall costs. Administration of fewer vaccines simplifies storage and delivery logistics, fewer staff are required for immunisation delivery, and the risk of immunisation related errors is reduced. Parents and immunisation providers have been shown to be reluctant for children to have multiple injections at an immunisation encounter. This may result in delayed completion of immunisations with increased risk to the infant of acquiring a vaccine preventable disease.

Provision of clinical data on immunogenicity and reactogenicity of new vaccines is required for licensing of new combination vaccines for children and young people in Australia and elsewhere. Combination vaccines trialled by our research unit and described in Chapter 1, are now available and incorporated in immunisation schedules around the world.

The impact of new combination vaccines on the childhood immunisation schedule has been substantial. Since the results of research studies assessing the various combinations of DTPa, HepB, IPV and Hib vaccine antigens have been presented, a hexavalent vaccine has been licensed in many countries so that infants now receive DTPa-HepB-IPV-Hib in one injection. However there is currently a limit on the number of antigens that can be mixed in the one syringe so it unlikely with current technology that all vaccines will be given in one injection.

Meningococcal combination vaccines

The excellent results of the Hib-MenCY vaccine study have led to a further study by our group to assess the safety and immunogenicity of Hib-MenCY vaccine co-administered with MMR vaccine. The results of this study have been pivotal in submission of the file to the FDA on 10 August 2009, for licensing of HibMenCY vaccine in the US. The vaccine dose for the more recent Hib-MenCY study was chosen from the study outlined in Paper *4*. The results of the primary component of this second study were presented at the 45th Annual Meeting of the Infectious Diseases Society of America (IDSA), San Diego, California, October 4-7, 2007 and the booster vaccination results have recently been presented by Prof Terry Nolan at the 49th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, California, 12-15 September 2009. A manuscript outlining the results of the study is soon to be submitted to the Pediatric Infectious Diseases Journal.

In the UK, a dramatic reduction in invasive Hib and meningococcal C disease has been observed following the introduction of Hib and meningoccocal C vaccines with the use of a combination HibMenC vaccine (also trialed by our research unit) as a booster at 12 months of age. A manuscript outlining the results of the Hib-Men C booster study is to be submitted for publication in the fourth quarter of 2009.

A combination vaccine to provide protection against all the various meningococcal subgroups (A, B, C, W, Y) causing invasive disease in humans is closer to becoming a reality. Since the success of the meningococcal C vaccine program in Australia, 90% of the remaining cases of invasive meningococcal disease are now due to meningococcal serogroup B. Our research unit was the first research group in the world to trial a novel meningococcal B vaccine, (LP2086, First In Man (FIM) study) based on recombinant outer membrane proteins to provide protection against endemic meningococcal B disease. Previous meningococcal B vaccines have been developed for epidemic disease due to a specific subtype causing disease in a region or country (MenNZ vaccine developed for epidemics in New Zealand). The usual

conjugation process to capsular proteins (used for conjugate A,C, W, Y vaccines) is unsuccessful for Men B vaccines as the Men B capsular proteins are very similar to human neural proteins which renders the subsequent vaccine poorly immunogenic with the concern that its use may precipitate autoimmune disease in the recipient. A study conducted in the 1960s showed vaccine made with Men B capsular proteins to be poorly immunogenic (antibodies were detected but non functional) but there was no increased risk of autoimmune disease identified.

This LP2086 vaccine has now been trialled in adolescents and toddlers by our research unit and other sites in Australia, with a worldwide Phase 3 clinical trial in adolescents planned for 2010. A manuscript outlining the Men B vaccine FIM study results is currently being prepared for submission for publication. I presented the results of the FIM and adolescent Men B vaccine studies at the International Pathogenic Neisseria Conference (IPNC), Rotterdam, The Netherlands in September 2008.

The changing epidemiology of the disease and recent deaths from invasive meningococcal B disease in Australia has heightened the importance of the development of an effective meningococcal B vaccine to protect against endemic disease.

We are awaiting results from a clinical trial conducted by our research unit of a combination MenACWY conjugate vaccine administered to toddlers. The ultimate goal is to develop a combination vaccine available to provide protection against all five meningococcal serogroups.

In Africa where endemic and epidemic Meningococcal A disease causes tens of thousands of deaths per year a conjugate meningococcal A vaccine is being trialled. The conjugate meningococcal A vaccine is likely to have the greatest impact on disease burden, due to the large number of cases of invasive meningococcal disease it will prevent.

Respiratory virus vaccines

Influenza vaccines

Vaccines to prevent respiratory viruses are still being developed and tested in clinical trials. The intranasal influenza vaccine discussed in Chapter 2 is licensed and available in the US and has been filed for licensure in Australia. Novel influenza virus vaccines are being developed as each influenza epidemic or pandemic occurs. Our research group has been involved in a clinical trial to assess the safety and

immunogenicity of a novel HIN1 vaccine in children in a combined Government, TGA and industry response to the current H1N1 pandemic. I am the Principal Investigator at our site for this expedited study to assess the safety and immunogenicity of novel H1N1 Influenza 09 vaccine in children prior to roll-out of the vaccine to the Australian community. Australia has been a world leader in responding to the current H1N1 epidemic with the manufacture, clinical trials and roll-out of a vaccine to the population to provide protection against H1N1.

Vaccines to prevent H5N1 have also been developed and stock-piled by Governments concerned about the mortality associated with previous pandemics.

Combination intranasal respiratory vaccines

RSV and PIV3 vaccines are considered a high priority by paediatricians, but a safe and effective combination vaccine has been elusive. PIV3-cp45 vaccine is expected to prevent several significant clinical syndromes commonly caused by PIV3, including acute otitis media, lower respiratory infection and febrile upper respiratory tract infection, but determination of efficacy will require further studies.

The addition of live attenuated RSV vaccine and, possibly, other live attenuated vaccines (PIV1, PIV2, and hMPV) would be a significant advance in controlling viral respiratory disease in young children, but as discussed in Chapter 2, for various reasons may not be available for several years.

A more recent advance in RSV-PIV3 vaccine development is the new experimental genetically engineered RSV-PIV3 intranasal vaccine. This chimeric vaccine contains a bovine PIV-3 backbone with human RSV F and G glycoproteins inserted into the PIV3 backbone. This vaccine has been shown to be immunogenic in preclinical studies. I am the Principal Investigator for Australasia for an immunogenicity and safety study of the genetically modified RSV/PIV3 intranasal vaccine, to commence in November 2009.

Community and immunisation provider acceptance of new vaccines

Community acceptance of new vaccination programs requires research to be conducted prior to introduction of the campaign to understand the knowledge of and concerns in the community about the introduction of new vaccines. Our unit has developed a strong research profile in this area, particularly in relation to community acceptance of HPV vaccines and pandemic influenza vaccines. Further studies

include a proposal to assess reasons for suboptimal uptake of HPV vaccine through a school based immunisation program. Australia is a world leader in establishing a funded immunisation program for HPV vaccine administered through schools. As our study results suggest (Paper 10), community support for the vaccine has in general been strong. However, adverse publicity in the media has resulted in concern amongst some adolescents and their parents about safety of the vaccine. In South Australia, one school opted out of the HPV immunisation program and did not offer vaccination though the school based program – this has not occurred with any other vaccine distributed through a school based program.

In the US, more recently concerns about HPV immunisation have been raised particularly amongst religious groups. Uptake of HPV vaccine has been lower than expected in comparison with other vaccines distributed through a school immunisation program with 77% of year 8 girls and 55% of year 12 girls receiving three dose of the vaccine compared to a 94% coverage rate in 18 month old children. Concern has also been raised about the uptake of other vaccines given concomitantly with HPV vaccine. Coverage for Hepatitis B vaccine given concomitantly with HPV vaccine in 2007 was only 72% compared to 82% in the preceding year.

Therefore, lower than expected acceptance and consent for immunisation with HPV vaccine may also have implications for other vaccines that historically have shown good uptake in adolescents. A pilot study to assess barriers to uptake of vaccine through a school based program is ongoing and has been funded by a University of Adelaide Faculty of Health Sciences grant and funding from SA Health. Data from this pilot study will contribute towards an ARC linkage grant proposal to be submitted in November 2009 to assess partnerships between education and health in achieving optimal outcomes for health service delivery through schools such as immunisation programs. I am also an invited member of a national HPV working party, headed by Dr Julia Brotherton, Epidemiologist, National HPV Vaccination Program Register to assess acceptance of HPV vaccine through research in the community.

Research into community knowledge and acceptance of PI preparedness has resulted in a successful collaboration with public health practitioners and the award of an NHMRC grant ($186,000) ID 626867 for research on the current H1N1 influenza pandemic. I am the Principal Investigator on the project entitled "Evaluating community understanding of and participation in strategies to prevent the spread of H1N1". The

study will be conducted in 2009 – 2010 with preliminary results to be presented by me at a national NHMRC workshop in Canberra in December 2009 to inform public health policy.

Vaccine safety

Monitoring the safety of vaccines remains an essential component of any new immunisation program, including post licensure surveillance of adverse events.

Due to the observed frequency in local injection site reactions following booster DTPa vaccine, the ATAGI recommended the removal of the DTPa booster vaccination at 18 months so that children receive only four DTPa vaccinations in the first 5 years of life. The reported studies indicate that a lower antigen dTpa vaccine may be less likely to give rise to large local reactions when used as a booster dose. However further studies are warranted to assist in determining the appropriate age for switching to reduced-antigen-content vaccines, i.e. to define when the benefits of reduced reactogenicity clearly outweigh a potentially lower immunogenicity.

In a further study being conducted by our research unit, ultrasound is being used to assess depth of vaccine deposition during vaccine administration. The study was considered feasible after the successful use of ultrasound in the extensive limb swelling study described above (Paper 14). This study has been designed to assess the appropriate angle for needle administration in infants, children, adolescents and adults comparing the depth of deposition of vaccinate when a needle is inserted at 60° or at 90°. The results of this study will contribute to immunisation policy regarding the appropriate angle of vaccine administration to ensure an intramuscular vaccination.

New vaccine schedules

Careful consideration must be given to an appropriate vaccination schedule for each vaccine as it becomes licensed. Post licensure surveillance data may indicate where schedules need to be amended due to adverse events or suboptimal long term immunity. Vaccination schedules should be flexible to allow changes to be made to improve acceptability and efficacy in provision of immunisations. As discussed in the presented papers is it likely that the pertussis vaccination schedule will see significant change in the near future in order to control epidemics. Ongoing research by our group includes a study to evaluate the severity of disease in children hospitalised with pertussis infection and validation of a severity score in

collaboration with nine other participating tertiary paediatric hospitals. The pertussis severity scoring system was designed by me with input from paediatricians and respiratory physicians from the Adelaide Women's and Children's Hospital, to accurately describe the impact of severe disease on children hospitalised with pertussis infection. This is a novel approach with no other pertussis severity scoring system available universally. We will also be assessing the impact of the current epidemic in South Australia. This information will be made available to the ATAGI pertussis immunisation working party.

The results of the pertussis vaccination at birth pilot study were central to the award of an NHMRC project grant No. 570756 ($1.45 million, CIA P McIntyre) for a larger study to examine neonatal pertussis immunisation at birth and to determine whether recent vaccination of mothers with a pertussis booster prior to pregnancy influences the antibody responses in the infant to the neonatal pertussis vaccination. We will be commencing this study in November 2009. The NHMRC study was designed by Prof Peter McIntyre from the National Centre for Immunisation Research and Surveillance with significant input from input from myself, Prof Terry Nolan, A/Prof Peter Richmond and Dr Nicholas Wood.

Following the outcome of combination Hepatitis A and B studies examining different schedules, a two dose schedule (adult formulation) is now offered as an alternative to the three dose paediatric formulation to provide protection for children against Hepatitis A and B infection.

Conclusion:

The contribution of this body of work to science and public health practice is significant. My research has included a broad approach to immunisation, ranging from the antibody and cellular immune response to vaccination to the mechanisms by which uptake of vaccination can be enhanced in the community. We need safe and effective vaccines but we also need acceptance and confidence within the community for an immunisation program to be successful. Our research group is one of two immunisation centres in Australia examining both the science of new vaccines and social implications of vaccine introduction in the community. My research portfolio includes NHMRC funded studies, other competitive grant funded studies and Industry funded studies. The papers included in this thesis have been presented at national and international meetings as indicated in the chapters and in the Appendix.

I hope that our future research portfolio will include research for communities most susceptible to infectious disease at an early age – our Indigenous Australian children who have the benefit of high immunisation coverage rates but do not receive immunisations in a timely fashion, the reasons for which remain unknown.

I conclude my thesis with a quote from the "Father of Vaccinology", Stanley Plotkin

"Thus all of us – vaccine developers, producers, public health practitioners and government officials – have our work cut out: to make sure that every person in the world who needs a vaccine receives it. Although this is a daunting task, we should take it up with joy, as the result will be diminished human misery."

Stanley Plotkin

Appendix A: Curriculum Vitae

CURRICULUM VITAE

Dr Helen Marshall

01 March 2009

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ACADEMIC QUALIFICATIONS:

SCHOOLING: Primary School: Girton Girls School
Secondary School: Pembroke College /Norwood High School

UNIVERSITY: University of Adelaide

DEGREES: MBBS University of Adelaide (1987)
DCH Women's and Children's Hospital / University of Adelaide (1998)
MPH University of Adelaide (2004)
Dissertation: *Uptake of varicella vaccine in South Australia - a cross-sectional survey of parental attitudes to varicella immunisation.*

COURSES/PROGRAMS

1. Colleague Development Program (CDP), University of Adelaide, 08 July – 25 September 2008

2. Good Clinical Practice for Clinical Research Investigators, Association of Clinical Research Professionals, 17th August 2007

3. Fifth Advanced Vaccinology course, Pasteur Merieux Foundation, Annecy, France, May 10-21, 2004

4. Human Papillomavirus Master Class, by invitation of Prof Margaret Stanley, Cambridge University, May 2006, Bangkok, Thailand

5. The National Health and Medical Research Council workshop on Clinical Trials, 2001, Adelaide, South Australia

6. Computer Courses: Computers and Word processing, Keyboarding, Power Point, Excel, Access

PRIZES / AWARDS:

PHERT Scholarship (2005-2006)
The inaugural Public Health Education and Research Trust Post Masters Scholarship. A cross-sectional study of parental attitudes to the introduction of HPV vaccine ($8 000 grant)

ANZAME UPJOHN Award for Medical Education (1987-88)
Preventative Medicine in Developing Countries – a cost effective program to reduce common illnesses in the children of Samoa.

PROFESSIONAL APPOINTMENTS:

2006-current	**Director, Paediatric Trials Unit** **Department of Paediatrics, Women's and Children's Hospital**
2003-2008	**Clinical Lecturer Level B** **Discipline of Paediatrics, University of Adelaide**
2005-2008	**Clinical Lecturer Level B** **Discipline of Public Health, University of Adelaide**
2000-2006	Senior Medical Officer, Paediatric Trials Unit, Department of Paediatrics, Women's and Children's Hospital
1997-2000	Research Medical Officer, Vaccine Research Unit Department of Paediatrics, Women's and Children's Hospital
1995-1998	Visiting Medical Officer Accident and Emergency Dept, Women's and Children's Hospital
1996-1997	Research Medical Officer Department of Pulmonary Medicine
1996-1997	Visiting Medical Officer Medical Administration, Royal Adelaide Hospital
1997	Medical Advisor to CSC (Computer Sciences Corporation) South Australian Health Commission
1995-1996	Casemix Medical Auditor Deloittes Touche Tohmatsu/South Australian Health Commission
1990-1993	Paediatric Medical Registrar Adelaide Children's Hospital
1989-1990	Registered Medical Officer Adelaide Children's Hospital
1988-1999	Medical Intern Royal Adelaide Hospital

PROFESSIONAL SOCIETIES:

Member of the Public Health Association of Australia (2003-)
Member of the Medical Defence Association of South Australia
Executive Member of the South Australian Immunisation Forum (2004-2008)
Member of the Australian Medical Association of Australia (2006-2008)
Member of the Association of Clinical Research Professionals (2007-)

TEACHING / TRAINING:

2007-current Lecturer, Health Sciences Honours students, Discipline of Paediatrics

2003-current Lecturer, 4th year Medical Students, Discipline of Paediatrics
2003-current Lecturer, Diploma in Child Health, Discipline of Paediatrics
2005-current Lecturer, Public Health Science students, Discipline of Public Health
2006-current Examiner 5th year medical students OSCI exams, University of Adelaide
1999-current Oral Assessor for entry into medicine/dentistry
1997-1999 Tutor in General Paediatrics – medical student teaching, years 2-5
2005-current Co-ordinator of Paediatric Trials Unit Seminars
2005-2006 Co-ordinator of Allergy, Immunology, Rheumatology and Vaccinology program
2008 Poster assessor, Research Expo 2008, University of Adelaide, 22nd July 2008

PEER REVIEW

Competitive Grant funding
NHMRC
Channel 7 Children's Research Fund

Journals
Paediatrics and Child Health
Vaccine
Annals of Paediatrics
Epidemiology and Infection

EDITORIAL REVIEW:
The Australian Immunisation Handbook 8th edition 2003, 9th edition 2007
The Australian Medicines Handbook 2003/2004/2005/2006

RESEARCH FUNDING

Competitive Grant Funding

2008	HPV Immunisation of children with immunosuppression. Boros C, Marshall H, Gold M. Arthritis Australia and SA Health	$ 37, 000
2009/ 2010	Neonatal pertussis immunisation. McIntyre P, **Marshall H,** T Nolan, N Wood NHMRC project grant	$1, 454,200
2008	Human Papillomavirus vaccine; why are adolescents opting out ? A Braunack-Mayer, **H Marshall,** M O'Keefe. Faculty of Health Sciences new collaborations grant University of Adelaide / SA Health	$22,000
2004	Does pertussis imunisation at birth provide earlier protection? McIntyre P, **Marshall H** Financial Markets Foundation	$150,000
2004	Uptake of varicella vaccine – a cross sectional survey of parental attitudes to nationally recommended but unfunded varicella immunisation. **Marshall H**, Ryan P, Roberton D South Australian Immunisation Coordination Unit, SA Health	$6,000
2004	Immunisation practice and implications for General Practitioner provision of a recommended, but non-funded vaccine. **Marshall H,** Ryan P, Roberton D, Baghurst P Dept of General Practice, University of Adelaide	$3,500
2006/ 2007	A cross-sectional study to assess parental attitudes to the introduction of human papilloma virus (HPV) vaccine to prevent a sexually transmitted disease and potentially protect against cervical cancer. **Marshall H**, Ryan P, Roberton D, Beilby J. South Australian Immunisation Coordination Unit, Dept of Health/Discipline of Public Health	$22,000

2008 **Long term follow-up study of pertussis immunisation at birth.** Marshall H, **Walker J, Evans S,** DeGaris L. Women's and Children's Hospital Foundation Research grant $18,000

Industry funding 2003 - 2008

2003 – 2006	Human Metapneumovirus study (**H Marshall**)	MedImmune
2004 – 2006	HPV vaccine study (**H Marshall**)	GlaxoSmithKline
2005 – 2006	RSV monoclonal antibody study (**H Marshall**)	MedImmune
2005 – 2007	HibMenCY vaccine study (**H Marshall**)	GlaxoSmithKline
2006	RSV monoclonal antibody crossover study (**H Marshall**)	MedImmune
2006 – 2007	Phase I Meningococcal B vaccine FIM study in adults (**H Marshall**)	Wyeth
2007 – 2008	Phase I Meningococcal B vaccine in toddlers (**H Marshall**)	Wyeth
2007 – 2008	Phase I Meningococcal B vaccine in adolescents (**H Marshall**)	Wyeth
2007 – 2008	Group A streptococcal carriage in children (**H Marshall**)	Wyeth
2007	HibMenC vaccine study (**H Marshall**)	GlaxoSmithKline
2007-2008	Meningococcal B vaccine blood collection study (**H Marshall**)	Wyeth
2007-2008	Meningococcal B duration of immunity study (**H Marshall**)	Wyeth
2008	Meningococcal A, C, Y, W vaccine study (**H Marshall**)	Sanofi-Pasteur
2008	Phase IV Adult influenza vaccine study (**H Marshall**)	CSL
2009	RSV/PIV3 intranasal vaccine in toddlers/infants **(H Marshall**)	MedImmune
2008	Meningococcal B vaccine adult blood collection study **(H Marshall**)	Wyeth
2008-2012	HibMenC long term follow-up (**H Marshall**)	GlaxoSmithKline
2009	Pandemic influenza vaccine in children (**H Marshall**)	GlaxoSmithKline
2009	Phase IV Paediatric flu vaccine safety study **(H Marshall)**	CSL
2009	RSV severity of hospitalized children study (**H Marshall**)	Abbott Australia
2009	Pertussis severity of disease in hospitalized children (**H Marshall**)	Sanofi-Pasteur
2009	Phase II Meningococcal B vaccine adolescent study **(H Marshall)**	Wyeth

PUBLICATIONS

1. **Marshall H**, Ryan P, Roberton D, Beilby J. **Varicella immunisation practice: Implications for provision of a recommended, but non-funded vaccine.** (accepted for publication 21 November 2008 **Journal of Paediatrics and Child Health**)

2. **Marshall H**, McIntyre P, Roberton D, Dinan L, Hardt K, Schuerman L. **Safety and immunogenicity of primary and booster immunization with a diphtheria, tetanus, acellular pertussis, hepatitis B (DTPa-HBV) and *Haemophilus influenzae* type b vaccine administered separately or together** (accepted for publication 02 March 2009 in **International J Infectious Disease**).

3. Gibson R, Barclay D, **Marshall H**, Moulin J, Maire J-C, Makrides M. **Safety of supplementing infant formula with long chain polyunsaturated fatty acids and Bifidobacterium lactis in term infants: a randomized controlled trial. British Journal of Nutrition** 2008;101:1-8

4. **Marshall H**, Isaacs D. **"I want the one for older women" – extending the human papillomavirus vaccine population base.** Letter to the editor. **MJA** 2008;189(9):527.

5. Nolan T, Bernstein D, Block S, Hilty M, Keyserling H, Marchant C, **Marshall H**, Richmond P, Yogev R, Cordova J, Cho I, Mendelman P for the LAIV Study Group. **Safety and Immunogenicity of Concurrent Live Attenuated Influenza Vaccine With Measles-Mumps-Rubella and Varicella Vaccines in Infants 12 to 15 Months of Age. *Pediatrics*** 2008;121:508-516.

6. Nolan T, Lambert S, Roberton D, **Marshall H**, Richmond P, Streeton C, Poolman J, Boutriau D. **A novel combined *Haemophilus influenzae* type-b-*Neisseria meningitidis* serogroups C and Y-tetanus-toxiod conjugate vaccine is**

immunogenic and induces immune memory when co-administered with DTPa-HBV-IPV and pneumococcal vaccine in infants. ***Vaccine*** 2007;25:8487-8499.

7. **Marshall H**, Ryan P, Roberton D, Baghurst P. **A cross sectional survey to assess community attitudes to introduction of HPV vaccine.** ***ANZ J of Public Health*** 2007;31(3): 235-242

8. Nolan T, Roberton D, **Marshall H,** Richmond P, Streeton C. **A combined DTPa-HBV-IPV vaccine for Australian infants.** ***J Paeds and Child Health*** 2007;43:576-81.

9. Peadon E, Burgner D, Nissen M, Buttery J, Zurynski Y, Elliot E, Gold M, **Marshall H**, R Booy. **Case for varicella surveillance in Australia**. Editorial Comment ***J Paeds and Child Health*** 2006;42:663-664.

10. **Marshall H,** Gold M, Roberton D, Gent R, Quinn P, Piotto L, Clarke M. **Ultrasound Examination of Extensive Limb Swelling Reactions After Diphtheria-Tetanus-Acellular Pertussis or Reduced-Antigen Content Diphtheria-Tetanus-Acellular Pertussis Immunization in Pre-school-Aged Children.** ***Pediatrics*** 2006;118(4):1-9.

11. Jacquet JM, Begue P, Grimprel E, Reinert P, Sandbu S, Silverdal SA, Faldella G, Nolan T, Lambert S, Richmond P, Roberton D, **Marshall H,** Schuerman L. **Safety and immunogenicity of a combined DTPa-IPV vaccine administered as a booster from 4 years of age: a review.** ***Vaccine*** 2006;24:2440-2448.

12. **Marshall H,** Nolan T, Roberton D, Richmond P, Lambert S, Jacquet J-M, Schuerman L. **A comparison of booster immunisation with a combination DTPa-IPV vaccine or DTPa plus IPV in separate injections, when co-administered with MMR at age 4-6 years.** ***Vaccine*** 2006;*24:6120-6128.*

13. **Roberton D, Marshall H, Nolan T, Sokal E, Diez-Domingo J, Flodmark C-E, Rombo L, Lewald G, de la Flor J, Casanovas J, Verdaguer J, Mares J, Van Esso D, Dieussaert I, Stoffel M. Reactogenicity and immunogenicity profile of a two-dose combined Hepatitis A and B vaccine in 1-11 year old children.** ***Vaccine*** **2005;*23(43):5099-5105.***

14. **Marshall H,** Ryan P, Roberton D. **Uptake of varicella vaccine – a cross sectional survey of parental attitudes to nationally recommended but unfunded varicella immunisation.** ***Vaccine*** 2005;23:5389-97.

15. D Roberton, H Marshall, L Dinan, C Boros, M Gold. Developmental immunology and vaccines. *Expert Rev. Vaccines* 2004;3(4):343-347.

16. D Isaacs, H Kilham, **H Marshall. Should routine childhood immunisations be compulsory?** ***J Pediatr Child Health*** 2004;40:392-396.

17. Belshe RB, Newman FK, Tsai TF, Karron RA, Reisinger K, Roberton D, **Marshall H**, Schwartz R, King J, Henderson FW, Rodriguez W, Severs JM, Wright PF, Keyserling H, Weinberg GA, Bromberg K, Loh R, Sly P, McIntyre P, Ziegler JB, Hackell J, Deatly A, Georgiu A, Paschalis M, Wu SL, Tatem JM, Murphy B, Anderson E. **Phase 2 Evaluation of Parainfluenza Type 3 Cold Passage Mutant 45 Live Attenuated Vaccine in Healthy Children 6–18 Months Old.** ***JID*** 2004;189(3):462-470.

18. Belshe RB, Newman FK, Anderson EL, Wright PF, Karron RA, Tollefson S, Henderson FW, Meissner C, Madhi S, Roberton D, **Marshall H**, Loh R, Sly P, Murphy B, Tatem JM, Randolph V, Hackell J, Gruber W, Tsai TF. **Evaluation of Combined Live, Attenuated Respiratory Syncytial Virus and Parainfluenza 3 Virus Vaccines in Infants and Young Children.** ***JID*** 2004;190:2096-2103.

Articles submitted for publication (2008/2009)

1. **Marshall H**, Ryan P, Roberton D, Street J, Watson M. Pandemic Influenza: Community Preparedness? (submitted to Am J Public Health Oct 2008)

2. Quinn P, Gold M, **Marshall H**, Royale J, Buttery J, Richmond P, McIntyre P, Wood N. Immunogenicity and reactogenicity of DTPa compared with dTpa vaccine administered to healthy children 4 to 6 years of age, having previously experienced injection site reaction following DTPa. (to be submitted to Pediatrics 2009)

3. White OJ, Rowe J, Richmond P, **Marshall H**, McIntyre P, Wood N, Holt PG. Cellular and humoral immune responses in infants to acellular pertussis vaccine administered at birth: randomized non-blinded pilot study. Tobe submitted to the BMJ

4. Wood N, McIntyre P, **Marshall H**, Roberton D. Pertussis in infants: can acellular pertussis vaccination at birth provide earlier protection? Submitted to J Paed Infect Disease 2009.

5. Adams J, Booy R, Buttery J, Elia S, Elliot E, Gold M, Heath C, McKay N, **Marshall H,** McIntyre P, Philips A, Pym M, Rhind L, Richmond P, Royle J, Wall K, Wood N, Zurynski Y. The development and trial of paediatric active enhanced disease surveillance (PAEDS): A new surveillance mechanism for Australia. ANZ J PH.

6. Booy R, Richmond P, Nolan T, **Marshall H**, Nissen M, Reynolds G, Ziegler J, Mesaros N, Boutriau D
Immunogenicity of a single dose of the combined *Neisseria meningitis, Haemophilus influenzae* type b-meningococcal serogroup C (HibMenC-TT) vaccine coadministered with MMR vaccine in 12-18 month-old Hib primed toddlers.

Papers published as part of a clinical study group

International Tacrolimus Ointment study Group
1. T Hofman, N Cranswick , P Kuna, A Boznanski, T Latos, M Gold, DF Murrell, K Gebauer, U Behre, E Machura, J Olafsson, Z Szalai, on behalf of the **International Tacrolimus Ointment Study Group**. Arch Dis child 2006;91(11):905-910.

Articles published (non-peer reviewed)

1**. Marshall H. Immunisation: A New Era For Vaccines.** Public Health Bulletin, edition 4, 2006.
2. Dugdale S, Lewis S, Gold M, **Marshall H**. **Vaccine Safety and Community Attitudes in SA**. Public Health Bulletin, edition 4, 2006.

Educational publications
1. **Marshall H.** Check Program : Immunisation 2004 Immunisation Up-date for General practitioners

ABSTRACTS

Oral and poster presentations at National and International Scientific meetings
1. **Marshall H**, Roberton D. Evaluation of PIV3 cp45 Parainfluenza Vaccine in Children Aged 6-18 Months. Public Health Association of Australia Immunisation Conference. Melbourne, Victoria, August 2002. (Oral presentation)

2. **Marshall H,** Roberton D, Nolan T, Sokal E, Diez-Domingo J, Flodmark C-E, Rombo L, Lewald G, de la Flor J, Casanovas J, Verdaguer J, Mares J, Van Esso D, Dieussaert I, Stoffel M. Reactogenicity and immunogenicity profile of a two-dose combined Hepatitis A and B vaccine in 1-11 year old children. Public Health Association of Australia Immunisation Conference, Melbourne, Victoria, August 2004. (Oral presentation)

3. **Marshall H,** Roberton D, Nolan T, Sokal E, Diez-Domingo J, Flodmark C-E, Rombo L, Lewald G, de la Flor J, Casanovas J, Verdaguer J, Mares J, Van Esso D, Dieussaert I, Stoffel M. Reactogenicity and immunogenicity profile of a two-dose combined Hepatitis A and B vaccine in 1-11 year old children. Advanced Vaccinology Course, Pasteur Merieux Institute, Anecy, FRANCE, May 2004. (Poster presentation)

4. **Marshall H**, Roberton D. Evaluation of Combined Live, Attenuated Respiratory Syncytial Virus and Parainfluenza 3 Virus Vaccines in Infants and Young Children. Public Health Association of Australia Immunisation Conference, Cairns, Queensland, August 2004. (Oral presentation)

5. Marshall H, Gold M, Roberton D, Gent R, Quinn P, Piotto L, Clarke M. Ultrasound Examination of Extensive Limb Swelling Reactions After Diphtheria-Tetanus-Acellular Pertussis or Reduced-Antigen Content Diphtheria-Tetanus-Acellular Pertussis Immunization in Pre-school-Aged Children. Public Health Association of Australia Immunisation conference, Cairns, Queensland, August 2004. (Oral presentation)

6. Marshall H, Ryan P, Roberton D. Uptake of varicella vaccine – a cross sectional survey to assess uptake of and parental attitudes to nationally recommended but unfunded varicella immunisation. Australian Society for Infectious Disease conference, Margaret River, Western Australia August 2005. (Oral presentation)

7. Marshall H, Ryan P, Roberton D. Uptake of varicella vaccine – a cross sectional survey to assess uptake of and parental attitudes to nationally recommended but unfunded varicella immunisation. Public Health Association of Australia annual conference, Perth, September 2005. (Oral presentation)

8. **Marshall H,** Ryan P, Roberton D, Baghurst P. A Cross-sectional Survey To Assess Attitudes To Introduction Of Human Papillomavirus Vaccine, Public Health Association of Australia Immunisation conference, Sydney, New South Wales, August 2006. (Oral presentation)

9. **Marshall H,** Ryan P, Roberton D, Baghurst P. A Cross-sectional Survey To Assess Attitudes To Introduction Of Human Papillomavirus Vaccine, 37th Public Health Association of Australia Annual Conference, Sydney, New South Wales, 25th-27th September 2006. (Oral presentation)

10. **Marshall H**, Roberton D, Skinner R, Rombo L, Dubin G and HPV vaccine study group. Safety and immunogenicity of Human Papilloma Virus 16/18 L1 vaccine in 10-14 year old girls. Public Health Association of Australia Immunisation conference PHAA Immunisation conference, August 2006. (Oral presentation)

11. Quinn P, Gold M, **Marshall H**, Royle J, Buttery J, Richmond P, McIntyre P, Woods N. Immunogenicity and Reactogenicity of dTpa Versus DTPa Vaccine in Children aged 4-6 years. Public Health Association of Australia Immunisation conference PHAA Immunisation conference, August 2006. (Oral presentation)

12. Guiso N, Cherry J, Ayala S, de Greeff S, Hallander H, Halperin S, Hogh B, Wirsing von Konig C, Lee B, Marchant C, **Marshall H**, Mertsola J. Pertussis prevention in 2006: issues, solutions and strategies. 8th International Symposium Saga of the genus Bordetella, Paris, FRANCE, 7th-10th November 2006. (Poster Presentation)

13. Nolan T, Lambert S, Roberton D, **Marshall H**, Richmond P, Streeton C, Poolman J, Boutriau D. A novel combined Haemophilus influenzae type b-Neisseria meningitides serogroups C and Y-tetanus-toxoid conjugate vaccine is immunogenic and induces immune memory when co-administered with DTPa-HBV-IPV and conjugate pneumococcal vaccines in infants. Annual meeting of the Pediatric Academic Societies (PAS), San Francisco, USA, April-May 2006.

14. Nolan T, **Marshall H**, Richmond P, Waite S, Mesaros N, Miller J, Boutriau. Immunogenicity and Safety of a Combined *Haemophilus influenzae* type b – *Neisseria meningitidis* serogroups C and Y – tetanus toxoid conjugate (HibCY) candidate vaccine. 45th Annual meeting of the Infectious Diseases Society of America – IDSA San Diego, California, October 4-7, 2007. (Poster Presentation).

15. Makrides M, Gibson R, **Marshall H.** Effect of an infant formula supplemented with probiotics and long chain polyunsaturated fatty acids (LCPUFA) on growth and immune markers of term infants: a randomised controlled trial. 40th Annual meeting of the European Society for Paediatric Gastroenterology Hepatology and Nutrition. May 9-12, 2007, Barcelona, Spain

16. **Marshall H**, Clarke M, Nolan T, Kefford M, Richmond P, Adams J, Nissen M, Lambert S, Booy. A successful partnership for immunisation research; the link between industry, academia and government. Clinical Research Excellence conference – CRX07, Melbourne, Australia, August 2007. (Poster Presentation).

17. Wood N, McIntyre P, **Marshall H**, Roberton D. Immunogenicity of birth and one month old acellular pertusis (Pa) vaccine. ECAAC, Boston, USA, August 2007.(Poster presentation)

18. **Marshall H,** P Ryan, D Roberton. A cross-sectional study to determine community knowledge about and attitudes toward the threat of pandemic influenza. World Society for Pediatric Infectious Diseases – WSPID, Bangkok, Thailand 18th November 2007.(Oral presentation)

19. White O, Rowe J, Richmond P, McIntyre P, Wood N, **Marshall H**, Holt P. Immunogenicity of neonatal pertussis vaccination. - Australasian Society of Immunology, Sydney, 2-6 December 2007.

20. Pym M, Adams J, Booy R, Buttery J, Elia S, Elliott E, Gold M, Heath C, **Marshall H**, McIntyre P, Phillips A, Rhind I, Richmond P, Royle J, Wall K, Wood N, Zurynski Y. Paediatric Active Enhanced Disease Surveillance (PAEDS): A twelve month trial of a new surveillance mechanism for Australia. RACP scientific meeting Adelaide May 2008

21. Wood N, McIntyre P, **Marshall H**, Roberton D. Immunogenicity of birth and one month old acellular pertusis (Pa) vaccine. RACP scientific meeting Adelaide May 2008

22. White O, Rowe J, Richmond P, McIntyre P, Wood N, **Marshall H**, Holt P. Cellular responses in infants to acellular pertussis vaccine administered at birth. Australasian Vaccines and Immunotherapeutics Development (AVID), Sydney, May 2008

23. **Marshall H**, P Ryan, D Roberton, J Street, M Watson. Pandemic Influenza Preparedness: is the community really prepared? 13th International Congress on Infectious Diseases, Kuala Lumpur, Malaysia 18 – 22 June 2008. Abstract no.3598

24. Clarke M, **Marshall H,** Evans S, Tidswell J, Lee S, Walker J, Heath C, Weber D, De Garis L. Effective recruitment strategies for Paediatric Vaccine Trials: a clinical trial site's perspective Clinical Research Excellence 2009, Brisbane, 7-9 August 2008.

25. Buttery J, Royle J, Bines J, Pym M, Adams J, Booy R, Elia S, Elliot E, Gold M, Health C, **Marshall H,** McIntyre P, Phillips A, Rhind I, Richmond P, Wall K, Wood N, Zurynski Y. Intussusception surveillance following rotavirus vaccine introduction: paediatric active enhanced disease surveillance (PAEDS). PHAA Immunisation conference Sept 2008

26. **Marshall H**, P Ryan, D Roberton, J Street, M Watson. Pandemic Influenza: Community preparedness? PHAA Immunisation conference, Surfers Paradise, Sept 2008

27. Walker J, Evans S, Chen R, Clarke M, Weber D, DeGaris L, Heath C, Tidswell J, Bourdon S, Lee S, **Marshall H.** Recruitment of subjects to clinical vaccine trials – what works and what doesn't? PHAA Immunisation Conference, Surfers Paradise, Sept 2008.

28. Street J, **Marshall H,** Braunack-Mayer A, Ryan P and the FluViews team. Community views on allocation of scarce resources: two methods, two answers. PHAA Immunisation Conference, Surfers Paradise, Sept 2008.

29. **Marshall H**, Nissen M, Richmond P Lambert S, Roberton D, Gruber W, Lockhart S, Arora A. A randomized, placebo-controlled, double-blind, Phase 1 trial of ascending doses of meningococcal group B rLP2086 vaccine in healthy adults. International Pathogenic Neisseria Conference IPNC, The Netherlands, September 2008.

32. Richmond P, **Marshall H**, Nissen M, Lambert S, Jones T, Gruber W, Arora A. A randomised, observer-blinded, parallel group, active control, Phase 1 trial of meningococcal serogroup B rLP2086 vaccine in healthy children and adolescents aged 8 to 14 years. International Pathogenic Neisseria Conference (IPNC), The Netherlands, September 2008.

33. White OJ, Rowe J, Richmond P, McIntyre P, Wood N, **Marshall H**, Holt P. Immune responses in infants to acellular pertussis vaccine administered at birth. 7th Louis Pasteur Conference on Infectious Diseases 11-13 Nov 2008 Paris France

34. Royle J, Zurynski Y, Booy R, Elliot E, Buttery J, **Marshall H,** Gold M, Richmond P, N, McIntyre P, Wood N , Rhind L, Pym M, Heath C, McKay N. A new mechanism for childhood conditions: Paediatric Active Enhanced Disease Surveillance (PAEDS). RACP Sydney 2009.

35. **HibMenC abstract cdcb**

36. Paeds surveillance seizures cdcb

37. varicella PAEDS

INVITED PRESENTATIONS (NATIONAL/INTERNATIONAL)

1. HPV Symposium in immunocompromised children CCRE, University of Sydney, NSW
2. **Control of pertussis: from the cradle to the grave**. World Vaccine Congress, Sydney October 13-15th 2008 (International)

3. Chair, Immunisation Symposium, PHAA Immunisation conference (speakers: Ian Fraser,) Surfers Paradise (National)

4. **Community perceptions of HPV vaccination**. HPV Vaccine Forum 8th August 2008, National Centre in HIV Epidemiology and Clinical Research, University of New South Wales. Garvan Institute, Darlinghurst, NSW (National).

5. **New Vaccines: technology, development, ethics and dilemmas** RACP Scientific Meeting 11-14th May 2008, Adelaide Convention Centre. (National)

6. **Parental attitudes to HPV immunisation** 34th Annual Scientific meeting of the Clinical Oncological Society of Australia, Prevention, Palliation and Cure: Progress through Clinical Trials, Plenary Session., 14-16 November 2007, Adelaide Convention Centre. (National)

PARTICIPATION IN GLOBAL ADVISORY BOARDS

2008	Paediatric Infectious Diseases Global Advisory Board, Paris Dec 2008 – Merck
2006	Global Advisory Board – Zoster vaccines – Sydney 2007-2008 - CSL
2005-2007	Global Advisory Board - MeaslesMumpsRubellaVaricella vaccine – Vienna, 2006 - Merck
2006	Independent Scientific Advisory Board meeting: Pertussis prevention in 2006: issues, solutions and strategies – Amsterdam 2007 - GSK.

Appendix B: Record of Medical Achievement

Record of Medical Achievement

I am a medical graduate of the University of Adelaide and have completed a Diploma in Child health (Department of Paediatrics, Women's and Children's hospital and University of Adelaide) and Master in Public Health degree (University of Adelaide). I completed the advanced Vaccinology Course in 2003 at the Pasteur Merieux Institute, France and have gained expertise in vaccinology, public health and social epidemiology. I was the recipient of the inaugural Public health Education and Research Trust Scholarship, awarded following completion of the Master in Public Health degree (2006). I recently completed the Association of clinical Research Professionals (ACRP) Good Clinical Practice Guidelines course for Investigators.

Present Occupation

- Director, Vaccinology and Immunology Research Trials Unit (VIRTU), Women's and Children's Hospital, Children, Youth and Women's Health Service
- Senior Lecturer (Clinical Title Holder), Discipline of Paediatrics, School of Paediatrics and Reproductive Health, University of Adelaide
- Senior Lecturer (Clinical Title Holder), Discipline of Public Health, School of Population Health and Clinical Practice, University of Adelaide

Academic Qualifications

- Bachelor of Medicine and Bachelor of Surgery, University of Adelaide, 1988
- Diploma of Child Health, Dept. Paediatrics, University of Adelaide, 1999
- Master of Public Health, University of Adelaide, 2004

Membership and Affiliations

- Member of the European Society of Infectious Diseases (2009 – current)
- Member of the Association of Clinical Research Professionals (2007- current)
- Member of the Public Health Association of Australia (2003 - current)
- Member of the Medical Insurance Group Australia (2004 – current)
- Executive Member of the South Australian Immunisation Forum (2004-2008)
- Member of the Australian Medical Association of Australia (2006-2008)

Leadership

In my role as Director of the Vaccinology and Immunology Research Trials Unit (formerly the Paediatric

Trials Unit) within the Discipline of Paediatrics, I provide leadership to a multi-disciplinary group of seventeen staff including three research medical officers, a research fellow, two senior researchers, five scientists and six specialist research nurses. As Director of VIRTU with almost 12 years experience in vaccinology research I have been the Principal Investigator or Co-Investigator for over 50 clinical trials and studies in epidemiology. These studies include Phase I – Phase IV safety, immunogenicity and efficacy trials in experimental vaccines. In addition, my research has included epidemiological studies of disease prevalence, community attitudes to the introduction of new vaccines and adverse events related to vaccination. The VIRTU is part of the Women's and Children's Hospital (WCH), a facility of the Children Youth and Women's Health Service (CYWHS). The research unit is affiliated with the University of Adelaide and is located within the Discipline of Paediatrics, School of Paediatrics and Reproductive Health. VIRTU is recognised nationally and internationally for its abilities to conduct clinical research to an excellent standard and in teaching and education in vaccinology. Over a ten year period over $4.5 million dollars in funding has been provided from both industry sponsorship and competitive grant funding.

VIRTU staff are trained in clinical trial conduct, International Conference in Harmonisation Good Clinical Practice (ICH GCP) guidelines and effective communication of research through abstract presentations and manuscript preparation. Continuing education includes monthly Vaccinology Research Seminars which I organise on topics relevant to clinical trials, infectious disease and vaccinology. These seminars, held in the Discipline of Paediatrics are widely published amongst hospital and university staff and presented by invited speakers for the benefit of staff and students. I supervise the training of VIRTU staff to ensure that staff are adequately trained and appropriately qualified to perform their relevant functions within VIRTU and approve standardised training procedures for VIRTU clinical research conduct. In addition, I supervise a staff development program which includes Good Clinical Practice (GCP) training, grant fund writing and manuscript preparation. As a review of the strategic direction for VIRTU, I organised and was a participant in a Strategic Planning Day held in October 2007. The meeting was facilitated by A/Prof Maree O'Keefe, Associate Dean of Learning and Teaching and achieved its goal of setting a 5 to 10 year strategic plan for our unit, the main goal being to improve success with competitive grant funding for academic research.

I am a supervisor for the World Health Organisation (WHO) Fellowship scheme for placement of Fellows with Australian clinical trials research units to gain experience in clinical trial conduct. This has involved supervision of two Paediatricians from China for a two week period and provision of a written assessment to the WHO. I have developed a program for visiting fellows covering clinical trials conduct

(VIRTU, WCH) and infectious diseases (Communicable Disease Control Branch (CDCB), Dept of Health; the Chest Clinic, Royal Adelaide Hospital).

Teaching

Medical/Health Sciences Teaching and Supervision Summary

- 2010 Medical student Year 4 MBBS Research Proposal
- 2009 PhD student (Co-supervisor 25%)
- 2008 Poster Assessor, Research Expo 2008, University of Adelaide, July 2008
- 2007-current Lecturer, Health Sciences Honours students, Discipline of Paediatrics
- 2003-current Lecturer, 4th year Medical Students, Discipline of Paediatrics
- 2003-current Lecturer, Diploma in Child Health, Discipline of Paediatrics
- 2005-current Lecturer, Public Health Science students, Discipline of Public Health
- 2005-current Co-ordinator Vaccinology and Immunology Research Trials Unit Seminar program
- 2005-2006 Co-ordinator of Allergy, Immunology, Rheumatology and Vaccinology program
- 2006-current Examiner 5th year medical students OSCI exams, University of Adelaide
- 1997-1999 Tutor in General Paediatrics – medical student teaching, years 2-5
- 1999-current Oral Assessor for entry into medicine/dentistry

Since graduation I have been involved in teaching medical students (years 2-5) and paediatric trainees in general paediatrics and clinical skills. More recently I have been involved in the teaching of vaccinology, research and clinical trial conduct and design to Medical, Public Health, Health Science and honours students. This has included the preparation and presentation of lectures based on material considered as cutting edge particularly in the area of vaccinology and investigational vaccines. I have employed a range of teaching methods including didactic presentations with use of video to demonstrate clinical examples, interactive problem solving and preparation of course notes with selected reference material for distribution to students. I have also set examination questions for the Diploma in Child Health examinations and fifth year medical student OSCI examinations. I have provided General Practitioner (GP) education sessions on new vaccines and lectures for the GP refresher weeks previously held at the WCH. These sessions have been formally assessed by completion of questionnaires by participants. The feedback I have received has been very positive. My goal in teaching is to develop interest and enthusiasm for learning, particularly in the importance of prevention of infectious disease.

I am a supervisor for 4th year medical students' research projects and I supervise health science students undertaking an honours year.

I completed the Colleague Development Program, University of Adelaide, in 2008.

Scholarship

Research Activity

During my twelve years as a researcher in vaccinology I have established four main areas of immunisation research and implementation of research outcomes to public health policy (outlined below). These research themes including 1. Investigational vaccines, 2. Vaccine Safety 3. Social Epidemiology/Health Economics of immunisation and 4. Infectious Disease Epidemiology are of critical importance to current research questions in vaccinology as well as key clinical and public health issues. I am a founding member of the National Immunisation Research Network, a group established to foster collaboration nationally amongst vaccinology research groups. I am considered nationally and internationally as an expert in vaccinology and have been invited to present scientific papers at both national and international meetings and critique papers submitted to both national and international journals in vaccinology and child health.

Investigational Vaccines

My most significant research has resulted from conducting clinical trials with investigational vaccines. Studies of investigational vaccines conducted in Australia provide local data to licensing and regulatory authorities. A significant contribution has been my involvement as Principal Investigator for the Meningococcal B vaccine project. The Meningococcus B strain is responsible for the majority of deaths from meningococcal infection in Australia and in many other countries such as the United Kingdom, the United States and Canada. Unfortunately a licensed Meningococcal B vaccine does not currently exist and is urgently required to reduce mortality from this infection. A novel vaccine has been developed and trialled initially in adults then adolescents, children and toddlers at our site. Our unit was approached by Wyeth (US) to conduct the First-In-Man (FIM) Phase I study with the novel Meningococcal B vaccine with subsequent testing in other age groups. Approval for the study to proceed in Australia was dependent on our site obtaining an external review of the planned protocol and study conduct from an international expert and subsequent ethics approval. The study results have indicated that this novel vaccine should be further developed and trialled for potential global use to prevent morbidity and mortality from meningococcal B infection. I presented the FIM and adolescent

study results at the International Pathogenic Neisseria Conference (IPNC) in The Netherlands in September 2008. A manuscript is currently being prepared on the FIM study results. The meningococcal B project continues in Australia with the help of collaborative colleagues (Prof Michael Nissan, Royal Children's Hospital, Brisbane, A/Prof Peter Richmond, Princess Margaret Hospital, Perth, Prof Terry Nolan, University of Melbourne, A/Prof Graham Reynolds, Royal Canberra Hospital, Canberra and Prof Robert Booy, Children's Hospital at Westmead, Sydney).

Other significant contributions include studies in new combination vaccines. This allows a reduction in the number of needles that need to be administered to infants and children making vaccination more acceptable (and more cost-effective with less time spent in the clinic) for all involved. Many of the new vaccine combinations trialled by our unit are now licensed (or have been filed for licensing) for use in Australian children including a combination measles, mumps, rubella, varicella vaccine. All studies have resulted in a publication or recent preparation of a manuscript for publication. I am responsible for ensuring all data resulting from clinical trials conducted by our unit are published whether the findings are positive or negative. Studies of investigational vaccines conducted at our site provide Australian data for regulatory authorities such as the Therapeutic Goods Administration (TGA) and Food and Drug Administration, (FDA, US). It is an advantage for both vaccine companies and the TGA to have safety and immunogenicity data based on the Australian population when licensing of a new vaccine is being considered.

Vaccine Safety

As the incidence of vaccine preventable disease has decreased, vaccine safety concerns have increased both within regulatory authorities and the community. Studies in vaccine safety identify adverse reactions associated with vaccination and provide data that support changes to the vaccination schedule to reduce the adverse effects associated with immunisation. Vaccine safety studies have included clinical and ultrasound assessment of extensive swelling reactions (ESR) following booster doses of diphtheria, tetanus and pertussis (DTPa) vaccine and comparison of low versus high antigen content DTPa booster vaccine in children with a previous ESR. Following the demonstration of acceptable immunogenicity the 18 month booster DTPa dose has been removed from the childhood schedule to reduce the incidence of extensive swelling reactions and promote compliance with childhood immunisation. A study of administration of pertussis vaccine at birth to provide earlier protection for infants from pertussis infection has provided important safety and immunogenicity pilot data to support an NHMRC grant application to investigate this novel change to the infant schedule.

Social Epidemiology

Community understanding and acceptance of vaccination is essential for high uptake of new vaccines and an effective immunisation campaign. I have been the lead investigator for several published studies examining community acceptance of new vaccines (varicella and Human Papillomavirus (HPV) vaccine) the data of which have been used by the Australian Technical Advisory Group on Immunisation (ATAGI) in making recommendations for varicella vaccine funding. In addition I have been invited to present the data on community acceptance of HPV vaccine at many meetings including GP education sessions and at the National Centre for Immunisation Research and Surveillance (NCIRS). The HPV vaccine study was funded by the grant I received as the inaugural recipient of the national Public Health and Education Research Trust Scholarship (PHERT). I have also been invited to present the data at HPV symposia interstate (New South Wales, Victoria, Queensland and Western Australia), having presented at the University of Adelaide HPV immunisation symposium held last year. This topic has also generated interest in the media and I have had several interviews with journalists resulting in articles quoting the positive results of this study. Data from a recent study examining the importance of community engagement in pandemic influenza preparedness was presented at the International Conference of Infectious Disease in June 2008 and was the first study published to detail community awareness and acceptance of government strategies to prevent spread of infection during an influenza pandemic. Research in this area is ongoing with my recent award of an NHMRC grant to investigate the current H1N1 Influenza 09 pandemic, investigating the community's response to government pandemic initiatives including school closures, to inform public policy.

Infectious disease epidemiology

In order to determine the most appropriate antigens to be incorporated into new vaccines and the best age for administration prior to exposure to infection, epidemiological data must be obtained. Group A streptococcal (GAS) infection is a cause of pharyngitis in children however in our indigenous population there is a high rate of immunological complications from GAS infection such as rheumatic heart disease and glomerulonephritis. The bacteria are also "carried" in the pharynx by asymptomatic children. A licensed vaccine to prevent GAS infection is urgently required particularly for our Indigenous community and I am the principal investigator for a study to asses the epidemiology of the infection including carriage in children aged 0-10 years. Data from this study will be used in the development of a potential GAS vaccine which will be trialled by our research unit. Human Metapneumovirus (hMPV) is a newly identified virus that causes severe lower respiratory tract infections (LRTI) in infants. I am the principal investigator for a study to assess the incidence of hMPV in infants with a history of prematurity presenting with a LRTI. These data will be used to determine whether development of a vaccine to

prevent this infection is feasible. In addition, I am the lead investigator of a national study to assess the severity of disease in infants and children admitted to hospital with pertussis and determine the disease burden in the current epidemic. These data will be provided to the ATAGI "pertussis working party" and presented at a public health immunisation conference to help in determining recommendations for the ideal pertussis immunisation program.

Grant Funding: details outlined in my CV (Appendix A)

DEST Category	*Category 1*	*Category 2*	*Category 3*
Funding	$555,000	$133,500	$1,852,069

I have been successful in obtaining grant funding from many sources to support my research, including three NHMRC grants, University of Adelaide grants, community funding organizations and Industry funding. I detailed list of funding sources and amounts is included in my CV (Appendix A).

Directorships/Board Membership

- Director, Vaccinology and Immunology Research Trials Unit (VIRTU), 2006 – current
- Meningococcal Leadership Forum, Istanbul, Turkey, 14-15th October 2009
- Consensus on pertussis booster vaccination in Europe (COPE), Brussels, Belgium, 9th June 2009
- Paediatric Infectious Diseases Global Advisory Board, Paris, France 10th Dec 2008
- Global Advisory Board, Zoster vaccines, Sydney 2007- 2008
- Global Advisory Board, Varicella immunisation, Vienna, Austria, May 2006
- Independent Scientific Advisory Board meeting: Pertussis prevention in 2006: issues, solutions and strategies. Amsterdam, The Netherlands, September 2007

Collaborations

I have long standing and highly productive collaborations locally, nationally and internationally. I collaborate with Dr Christina Boros, Dr Patrick Quinn and Dr Michael Gold within the Discipline of Paediatrics on vaccine related studies. Within South Australia collaborators include Mrs Maureen Watson, Director of the South Australian Immunisation Coordination Unit, Prof Philip Ryan, Discipline of Public Health and A/Prof Peter Baghurst from the Public Health Research Unit. Nationally I collaborate

with Dr Peter Richmond, Prof Terry Nolan, Prof Peter McIntyre, Prof Robert Booy and Prof Michael Nissen, all founding and co-members of the National Vaccine Research Network. Internationally I collaborate with the Medical Directors of the Research and Development Division of MedImmune (US), GlaxoSmithKline (Belgium), SanofiPasteur (US) and Wyeth (US).

Publications

I have published 21 research publications in peer-reviewed journals with high impact factors and a further 6 papers have been submitted for publication to date. I have presented study results at national and international public health, immunisation and infectious diseases meetings, a list of which is included in my CV.

Creative Activity / Consultancy

I have been invited to participate in several Global Advisory Boards with the aim to improve global immunisation coverage. Previous advisory boards have included a forum to discuss improving whole of life protection against pertussis (whooping cough) infection, improving coverage with varicella vaccine by introduction of a combination measles, mumps, rubella, varicella vaccine and a new varicella vaccine to prevent herpes zoster (shingles). I recently participated in an International Meningococcal Leadership Forum in Turkey to discuss the implications of the development of vaccines to cover all serogroups causing disease in humans. I have been an invited speaker at national and international conferences. I have been involved in several conference organising committees including a member of the scientific committee for the Clinical Research Excellence conference held in Brisbane in October 2008 and the Public Health Association of Australia conference to be held in Adelaide in august 2010. I recently established a Scientific Advisory Board (SAB) to provide strategic direction to VIRTU. The SAB is composed of international experts in paediatrics (Prof Geoff Davidson), vaccinology (Dr Peter Richmond), infectious disease (Prof David Isaacs) and public health (Prof Philip Ryan).

Professional Activity including service to the community

As a researcher within a tertiary teaching hospital with university affiliation I believe that I have an obligation to the community to provide education and understanding of current research within the WCH and the University of Adelaide. As a clinician and authority on immunisation I also have the responsibility to provide accurate and up-to-date information on immunisation issues to the community and to colleagues.

Professional Activity

I am a regular reviewer for Paediatrics in Child Health, (the prominent national journal for research in child health), Archives of Disease in Childhood (international journal in child health) and Vaccine (the primary international journal for publication of research in vaccinology). I have been approached by assessors to provide advice on several NHMRC grants involving projects in immunisation. Editorial responsibilities also include annual review of the vaccines chapter of the Australian Medicines Handbook and the Australian Immunisation Handbook. I was an active member of the South Australian Immunisation Forum (SAIF) which has now been disbanded but continue in an advisory capacity to the South Australian Immunisation Co-ordination Unit within the South Australian Department of Health. I am a regular attendee at the weekly Women's and Children's Hospital (WCH) medical rounds and presenter at the WCH and Flinders Medical Centre grand rounds. As a member of the Public Health Association of Australia (MPHAA) I regularly attend the PHAA annual conference and biannual PHAA Immunisation conference at which I regularly present scientific papers. I have recently chaired an Influenza Symposium held in Adelaide. I have been a faculty oral assessor for entry into medicine and dentistry since 1999 and was previously involved in the medical student mentoring program.

Community Service

Within the community I provide immunisation up-dates for general practitioners, the Child and Youth Health Service and GP refresher weeks previously held at the WCH. I have regularly given interviews for print/radio/TV media relating to current research in immunisation particularly new vaccines. These interviews have resulted in articles in national newspapers and weekly medical papers such as "Medical Observer" and "Australian Doctor". I am regularly invited to present on immunisation topics at GP education seminars held within South Australia and interstate. I was invited by the Royal Australian College of General Practitioners to complete an up-date on immunisation for the Check program, a monthly publication including self-assessment produced by the college. This teaching aid is based on problem solving and self-assessment. I have been invited to give presentations on immunisation at the Women's and Children's Hospital Public Health Seminar program and have produced a regular newsletter for distribution within the community describing the current research activities of VIRTU. I regularly attend school student career nights to discuss a career in medicine but more importantly to raise awareness of the importance of research in any medical career and the role of the academic physician. I have participated in the University of Adelaide Open Days for the Discipline of Paediatrics where I have provided information to prospective local and interstate medical students.

SUMMARY

I am a nationally and internationally recognised innovative researcher in vaccinology and public health. I am highly motivated with excellent organisational and communication skills and have developed sound research ability. I have a well established publication record and have received invitations to present my research at national and international meetings. I have the respect of the VIRTU staff as a fare, consistent, motivational and considerate leader. My achievements in research, teaching, professional activities and community service as described in this document have all been achieved within the framework of an 0.7 FTE appointment.

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