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## Serious adverse events after measles-mumps-rubella vaccination during a fourteen-year prospective follow-up

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**Background.** Several disorders have been attributed to measles-mumps-rubella (MMR) vaccination during the past decade. The aim of this prospective follow-up study was to identify serious adverse events causally related to MMR vaccination.

**Methods.** When the MMR vaccination program was launched in Finland in 1982, a countrywide surveillance system was set up to detect serious adverse events associated with MMR. To obtain detailed case histories vaccinees' clinical charts were reviewed. Serum samples were analyzed to trace concurrent infections.

**Setting.** All hospitals and health centers in Finland from 1982 through 1996.

**Results.** Immunization of 1.8 million individuals and consumption of almost 3 million vaccine doses by the end of 1996 gave rise to 173 potentially serious reactions claimed to have been caused by MMR vaccination. In all, 77 neurologic, 73 allergic and 22 miscellaneous reactions and 1 death were reported, febrile seizure being the most common event. However, 45% of these events proved to be probably caused or contributed by some other factor, giving an incidence of serious adverse events with possible or indeterminate causal relation with MMR vaccination of 5.3 per 100 000 vaccinees or 3.2 per 100 000 vaccine doses.

**Conclusions.** Causality between immunization and a subsequent untoward event cannot be estimated solely on the basis of a temporal relation. Comprehensive analysis of the reported adverse reactions established that serious events causally related to MMR vaccine are rare and greatly outweighed by the risks of natural MMR diseases.

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## INTRODUCTION

A combined vaccine comprising attenuated live measles, mumps and rubella (MMR) viruses was added to the schedule of voluntary and free of charge childhood immunization in Finland in November, 1982. Since then children have been vaccinated twice, at 14 to 18 months and 6 years of age. This comprehensive program has proved highly successful, and MMR diseases and their severe consequences have become rarities. As a result vaccine safety has become a more important issue than when these diseases were rampant.

With the launching of the vaccination policy, a countrywide prospective surveillance system was set up to clarify the incidence and nature of all serious events after MMR vaccination during 1982 through 1996. The aim was to distinguish events having a causal relation with MMR vaccination from those with only a temporal relation. We here report the results of >14 years of follow-up.

## METHODS

The passive surveillance system was launched by ad hoc personnel and a follow-up committee operating under the auspices of the National Board of Health and the National Public Health Institute.<sup>1</sup> A primary aim was to gather information about the incidence and nature of all the severe adverse events in a causal association with MMR vaccination. The surveillance system was designed to identify all such events that were too rare to have been detected by a double-blind crossover study conducted on 1200 twins.<sup>2</sup>

It was anticipated that potentially serious and unpredictable events would be reported more reliably than the commonplace reactions.

**Notification system.** All reports were sent to the central office by health care personnel, public health nurses, general practitioners or pediatricians in primary care and hospitals, who were provided with detailed information in a series of seminars held around the country before the project started.<sup>1</sup> All information was distributed in written form in Finnish and Swedish, the other official language. The public was informed by the media,<sup>3</sup> and several articles on the MMR project appeared in Finnish medical publications during the subsequent years.

Once a report arrived it was evaluated, and if needed the hospital or health center treating the vaccinee was contacted.

**Definitions.** A potentially serious adverse event was defined as an event in any temporal association (no time limit was imposed) with MMR vaccination that fulfilled one or more of three characteristics: a potentially life-threatening disorder (e.g. anaphylaxis); possibility that a chronic disease had been triggered by the vaccination (e.g. rheumatoid arthritis, diabetes); or the vaccinee had been hospitalized for reasons possibly

attributable to MMR vaccine. In case of such an event the first part of a special two part form was completed and mailed to the central office, whenever possible with a serum sample. The second part of the form and a second serum sample were sent 2 to 3 weeks later.

To facilitate reporting the forms with detailed instructions, tubes for serum samples, and prestamped padded envelopes were distributed to the 1000 child health centers and relevant hospitals.

Categorization of the cases (death, likely allergic reactions, neurologic disorders and miscellaneous events) was conducted by two of the authors (HP, specialist in pediatric infectious diseases, and AP). Likely allergic reactions were anaphylaxis,<sup>4</sup> urticaria<sup>5</sup> (sometimes accompanied by angioedema<sup>6</sup>), asthma,<sup>7</sup> Henoch-Schönlein purpura<sup>8</sup> and Stevens-Johnson syndrome.<sup>9</sup>

Neurologic disorders were divided into seizures (febrile seizures,<sup>10</sup> epilepsy,<sup>11</sup> and undefined seizures which did not meet any particular criterion and remained without accurate diagnosis), encephalitis,<sup>12</sup> meningitis,<sup>13</sup> Guillain-Barré syndrome,<sup>14</sup> gait disturbance and confusion during fever. A few cases of transient gait disturbance have been described before,<sup>15, 16</sup> but exact definition is lacking, as in the case of short lasting confusion during fever.

Finally the miscellaneous syndromes comprised pneumonia,<sup>17</sup> orchitis<sup>18</sup> and diabetes mellitus.

Judgment of the causal association with MMR vaccination was based on the clinical information obtained from medical records and notification forms and analysis of the serum samples. The incubation periods of measles (8 to 12 days), mumps and rubella (both 16 to 18 days)<sup>19</sup> were also used in the assessment of whether a nonallergic event was likely or unlikely to have been triggered by MMR vaccination. The incubation periods could not be applied to allergic events or those with unresolved etiology.

**Serology.** Serum samples were collected to trace possible concurrent infections manifesting with symptoms or signs mimicking those caused by vaccination. All samples were stored at -20°C until analyzed in 1998. Antibodies against *Haemophilus influenzae*, *Moraxella catarrhalis*, pneumococcus and adeno-, entero- and human parvoviruses were detected by enzyme immunoassay, whereas microimmunofluorescence was used for *Chlamydia pneumoniae* and indirect immunofluorescence for human herpesvirus 6. Details of the methodology are described elsewhere.<sup>20-24</sup>

**Vaccine and vaccinees.** M-M-R<sup>®</sup><sub>II</sub> (Merck & Co., Inc., West Point, PA), distributed as Virivac in Scandinavia, has been the only vaccine used in Finland except for the 2570 doses of Triviraten (Swiss Serum and Vaccine Institute, Berna, Switzerland) that were ad-

ministered to individuals with severe hypersensitivity in 1992 through 1996.

M-M-R<sup>®</sup><sub>II</sub> contains the more attenuated Enders-Edmonston strain of measles virus, the Jeryl Lynn B strain of mumps virus and the Wistar RA 27/3 strain of rubella virus. It also contains 25 µg of neomycin and traces of sorbitol and hydrolyzed gelatin. The vaccine is administered subcutaneously into the buttock (at age 14 to 18 months) or upper arm (subsequently).

Besides the main target groups, children at the age of 14 to 18 months and 6 years, intermediate age groups were immunized in various catch-up programs during the early years of the project.<sup>1, 25</sup> Recruits of the Defense Forces were included in 1986, whereas health care workers, nursing school students and once only vaccinated 11- to 13-year-old girls have been vaccinated since 1988. From 1988 to 1993 rubella-seronegative women were vaccinated after delivery. During outbreaks<sup>3</sup> vaccination was extended to unvaccinated adolescents.

## RESULTS

The reports of potentially serious adverse events were categorized as death, likely allergic reactions, neurologic disorders and miscellaneous events.

However, the majority of the reports concerned innocuous symptoms and signs not fulfilling the above mentioned criteria and were excluded from further analysis (Table 1). Idiopathic thrombocytopenic purpura was also excluded because it has been analyzed previously.<sup>26</sup>

**TABLE 1.** Reported minor or self-limited adverse events among 437 vaccinees\*

Sign or Symptom	Reported as	
	Main event†	Additional event‡
Fever	180	97
Rash	132	30
Lymphadenopathy	69	16
Rhinitis	37	27
Irritability	35	16
Cough	35	27
Conjunctivitis or photophobia	23	9
Local irritation or erythema	21	
Sore throat	20	8
Otitis media	19	9
Headache	14	6
Transient arthralgia	12	12
Diarrhea	10	8
Gingivostomatitis	7	
Swelling of parotid glands	6	3
Nausea or vomiting or both	5	17
Neck pain or stiffness	3	3
Fatigue	2	
Hepatosplenomegaly	2	
Abdominal pain	1	4
Pallor	1	
Sneezing	1	1
Sinusitis		2

\* If multiple events, all listed.

† Among 268 vaccinees, excluded from further analysis.

‡ Among 169 vaccinees, reported in conjunction with a potentially serious event (described in the text).

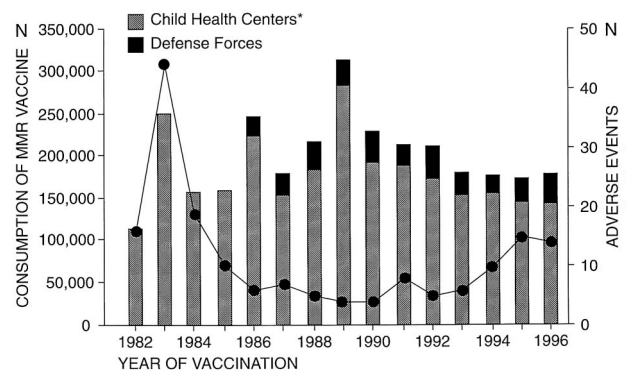
The vaccination coverage has oscillated around 95%.<sup>25, 27</sup> By the end of 1996 2 990 000 vaccine doses had been distributed for 1.8 million vaccinees. During the entire 14-year period 437 vaccinees were reported to have a vaccine-associated untoward event (Table 1); 173 reactions among 169 vaccinees, of whom 79 (46.7%) were hospitalized, were considered potentially serious. These 173 events were further scrutinized, except for a possible anaphylactic reaction in a 6-year-old child, whose medical records were not traced. Paired sera were available from 83 and a single sample from 19 of these vaccinees.

As might be expected, more events were reported soon after the beginning of the project; a clear peak was observed in 1983 (Fig. 1). However, 43% ( $n = 19$ ) of these 44 reports concerned febrile seizures. The reporting activity thereafter remained fairly static at 5 to 15 reports per year.

The age at the time of vaccination ranged from 13 months to 23 years; 57% of vaccinees were male and 43% were female. Simultaneous immunizations had been administered to 35 (20.7%) individuals; *H. influenzae* type b conjugate vaccine to 22, inactivated or live poliovirus vaccine to 9 and meningococcal polysaccharide vaccine to 1. In addition, 2 were vaccinated concurrently against tetanus, diphtheria, polio and meningococcal disease and 1 against *H. influenzae* type b and polio.

As illustrated in Fig. 2, the events developed in 2 peaks, within the first 24 h and on Days 7 to 10 postvaccination; 34% of cases occurred in each period ( $n = 59$  and 60, respectively). Most events, 81% ( $n = 140$ ), followed the first vaccination dose, but 16% ( $n = 28$ ) occurred after revaccination [dose not stated in 5 reports (3%)]. An exception to this were the suspected anaphylactic reactions, 63% ( $n = 19$ ) manifesting after the second dose.

**Serious adverse events. Death.** A previously healthy 13-month-old boy died during sleep 8 days after MMR vaccination. The parents had noticed tran-



**FIG. 1.** Vaccine doses distributed and serious adverse events reported annually. \*, including school health care centers, occupational health service and hospitals.

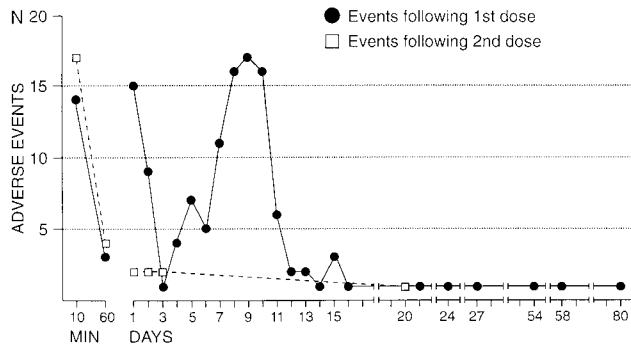


FIG. 2. Temporal distribution of the serious adverse events (time elapsed from vaccination to diagnosis). Six cases were not included because of incomplete information.

sient flaccidity and faintness a few hours preceding the death, but the symptoms had subsided immediately, and the boy had seemed entirely healthy when put to bed. Forensic autopsy disclosed the cause of death as aspiration of vomit caused by acute gastritis. His older sister also had a history of flaccid attacks unrelated to vaccinations.

**Allergic events.** Thirty suspected cases of anaphylaxis were reported, at age from 15 months to 23 years, only 2 boys were younger than 5 years. Epinephrine or corticosteroids had been administered to only 18 of these 30 vaccinees, and in 15 of the 30 cases the physician ultimately diagnosed fainting, based on lack of other signs of hypersensitivity, evident fear before vaccination, a history of syncope after other vaccinations or blood tests or a full and rapid recovery without medication. All reactions appeared within 20 min of vaccination, except in a 20-year-old woman, who developed shortness of breath, dizziness, diarrhea and facial angioedema several hours after vaccination, shortly after a spicy meal. Allergy tests were never conducted, but allergy to spice was suspected. Full recovery within 1 h, and usually within a few minutes, was the rule.

Urticaria occurred in 30 vaccinees, in 12 cases accompanied by angioedema. The symptoms appeared within a few minutes to 15 days. The age at vaccination ranged from 13 months to 23 years.

Asthma-like symptoms and signs commencing 10 min to 13 days after vaccination were reported in 10 vaccinees at the ages of 17 months to 17 years. Asthma had previously been diagnosed in 2 vaccinees, and egg allergy had been diagnosed in 2. Symptoms appeared in conjunction with upper respiratory tract infection in 4 children, 3 of whom had histories of similar episodes.

A 2-year-old girl developed Henoch-Schönlein purpura 3 days after MMR vaccination. A 6-year-old boy was also diagnosed with this disease 24 days postvaccination, but a verified streptococcal tonsillitis preceded the diagnosis by a few days. Both children recovered completely.

A 16-year-old boy developed Stevens-Johnson syn-

drome 4 days postvaccination, and 6 relapses with milder manifestations occurred during the following 7 years. Respiratory infections with reappearance of labial herpes simplex lesions were observed in connection with most of the relapses.

**Neurologic disorders.** The events most commonly reported were febrile seizures, occurring in 52 vaccinees 12 h to 15 days after vaccination. Apart from 3 children at the ages of 3 to 6 years, all were <3 years old. In a 14-month-old girl with a febrile seizure occurring 10 days postvaccination, serology detected influenza A infection. Tympanocentesis revealed *H. influenzae* and pneumococcal infections in 2 children with concomitant otitis media.

Epilepsy was diagnosed in 2 boys and a girl, all 6 years old. Symptoms manifested for the first time 1, 10 and 21 days postvaccination. One of the boys was later diagnosed as having severe Lennox-Gastaut syndrome, whereas medical records subsequent to the acute phase were not available for the other. The electroencephalogram of the girl normalized during follow-up and medication was discontinued.

Undefined seizures were observed in 4 girls 2 to 12 days postvaccination. Histories of breath-holding spells in 2 girls were noted. Brief convulsions were reported in the 14- and 23-month-old girls. The other 2 vaccinees, 21 months and 7 years old, underwent transient loss of consciousness, accompanied by fever in the older child. All seizures subsided without medication or sequelae. An electroencephalography was conducted in 2 children and lumbar puncture was performed in 1; all results were normal. One of the events was deemed by a neurologist to have been a breath-holding spell.

Of the four cases of encephalitis reported, the first has been described earlier;<sup>28</sup> acute lymphoblastic leukemia was diagnosed in a 6-year-old girl 23 days after MMR vaccination. During immunosuppressive treatment she developed severe measles encephalopathy 54 days postvaccination and interstitial pneumonia a few days later. In 1998, 14 years later, she had completed comprehensive school, and the leukemia had not relapsed, but she suffered from severe epilepsy. No factor indicated a causal connection between MMR vaccination and leukemia, because the symptoms that led to the diagnosis, pain in the extremities and back, were already present at the time of vaccination.<sup>28</sup>

Laboratory confirmation of herpes simplex encephalitis developing 6 days postvaccination in a 14-month-old boy indicated only a temporal association between vaccination and encephalitis. In contrast in 2 girls at the ages of 15 and 18 months who developed encephalitis 9 and 13 days postvaccination, a causal relation could not be excluded because no specific etiology was detected.

Meningitis was reported in 4 children. *H. influenzae* and meningococcal meningitis were diagnosed in a 15-month-old boy and 14-month-old girl 2 and 7 days postvaccination, respectively. In 2 boys 6 and 7 years old, no agent was detected. Both developed symptoms 2 days after the second MMR vaccination. The very short interval between vaccination and onset of the disease almost certainly excludes a causal association with MMR vaccination.

Guillain-Barré syndrome was diagnosed in two 18-month-old boys 10 and 27 days after immunization. Both soon recovered without complications. A causal association with vaccination cannot be excluded in these cases.

Transient gait disturbances were reported in five vaccinees, four boys and a girl 14 to 18 months old. The only visible sign was enlargement of the inguinal lymph nodes in one child; fever was present in three children. The symptoms manifested 2 to 8 days postvaccination and subsided within a few days.

Confusion during fever was reported in 3 boys 18 months to 2 years old. This nonspecific sign developed 2 to 9 days postvaccination and subsided spontaneously within a few hours.

No cases of autism<sup>29</sup> were associated with MMR vaccination during this 14-year follow-up.

**Miscellaneous complaints.** Pneumonia occurred 3 to 58 days postvaccination in 12 vaccinees 15 months to 6 years old. In 1 case pneumonia was caused by aspiration during a febrile seizure. Concomitant otitis media, caused by *H. influenzae* and by *M. catarrhalis*, was diagnosed in 2 boys by tympanocentesis.

In four 17- to 18-month-old boys, orchitis was suspected 5 to 9 days postvaccination. Three additional cases were reported, but they turned out to be swelling of the scrotum caused by urticaria without involvement of the testicles, testicular cancer and scrotal hernia, at the ages of 16 months, 17 months and 4 years, respectively. The cancer was diagnosed 16 days postvaccination and had not relapsed after operative treatment when checked 5 years later.

Diabetes mellitus was reported in three children. A 15-month-old girl was healthy when vaccinated, but polydipsia, fever, vomiting and diarrhea were noticed a couple of days later. The symptoms subsided 6 days postvaccination but recurred on the eighth day, leading to hospitalization and diagnosis.

Polydipsia and polyuria were observed 7 days after MMR vaccination in a 6-year-old boy in whom diabetes was diagnosed 1 week later. Another boy, 19 months old when vaccinated, developed symptoms insidiously over weeks until the diagnosis was made 80 days postvaccination.

The incidence of type I diabetes in Finland is the highest worldwide; ~30 new cases are expected in children aged 1 to 6 years during any 80-day period (J Tuomilehto, personal communication, 1998).<sup>30</sup> Because this is 10 times more than the incidence of diabetes found in this series, we deem a causal relation very unlikely.

No cases of ulcerative colitis, Crohn's disease or any other chronic disorder affecting the gastrointestinal tract<sup>29</sup> were reported.

**Documented causative or contributing factors.** An infectious agent or other factor not related to MMR

**TABLE 2.** Characteristics of vaccinees with verified concomitant infections

Agent	Reported Event	Age at Vaccination	Gender	Additional Symptoms	Dose
Pneumococcus	1. Pneumonia	6 yr	F	Fever, abdominal pain	2
	2. Febrile seizure	15 mo	M	Fever	1
	3. Anaphylaxis	5 yr 3 mo	M	Fever, sore throat	1
	4. Anaphylaxis	3 yr 4 mo	M	Otitis media	1
	5. Anaphylaxis	5 yr 11 mo	M		2
	6. Urticaria	13 yr	F	Angioedema, fever, vomiting, conjunctivitis	?
	7. Urticaria	18 mo	F	Lymphadenopathy	1
HHV 6	1. Febrile seizure	18 mo	M	Fever, cough, rhinitis	1
	2. Febrile seizure	15 mo	F	Fever	1
	3. Febrile seizure	2 yr 1 mo	F	Fever, rhinitis	1
	4. Febrile seizure	15 mo	M	Fever	1
	5. Febrile seizure	17 mo	F	Fever, cough, rhinitis	1
	6. Pneumonia	18 mo	M	Fever, rash, lymphadenopathy	1
<i>Moraxella catarrhalis</i>	1. Shortness of breath	20 mo	M	Rash	1
	2. Febrile seizure	17 mo	F	Fever, cough, rhinitis	1
	3. Febrile seizure	16 mo	M	Fever, cough, rhinitis, conjunctivitis, tonsillitis	1
Enterovirus	1. Anaphylaxis	6 yr	F		1
	2. Undefined seizure	23 mo	F		1
	3. Epileptic seizure	6 yr	F		1
<i>Haemophilus influenzae</i>	1. Urticaria	4 yr 2 mo	F		1
Pneumococcus + <i>Chlamydia pneumoniae</i>	1. Urticaria	6 yr	M	Angioedema, fever	1
Pneumococcus + <i>M. catarrhalis</i>	1. Pneumonia	6 yr	F	Fever, cough, rash	2
Pneumococcus + enterovirus	1. Pneumonia	16 mo	F	Fever, cough, rhinitis, lymphadenopathy, arthralgia	1
<i>M. catarrhalis</i> + enterovirus	1. Urticaria	18 mo	M	Cough, rhinitis	1
<i>H. influenzae</i> + enterovirus	1. Febrile seizure	18 mo	F	Fever	1

HHV 6, human herpes virus 6.

**TABLE 3.** Assessment of causality between MMR vaccination and 173 serious events

Entity	Reports ( <i>n</i> )	Association with MMR Vaccination			
		Not causal ( <i>n</i> )	Possibly causal		
			<i>n</i>	%	Incidence/ 100 000 doses
Death ( <i>n</i> = 1)	1	1	0	0	0
Likely allergic disorders ( <i>n</i> = 73)					
Anaphylaxis	30	16	14	47	0.5
Urticaria	30	5	25	83	0.8
Asthma	10	5	5	50	0.2
Henoch-Schönlein purpura	2	1	1	50	0.03
Stevens-Johnson syndrome	1	0	1	100	0.03
Neurologic disorders ( <i>n</i> = 77)					
Seizures					
Febrile seizure	52	24	28	54	0.9
Epilepsy	3	2	1	33	0.03
Undefined seizure	4	2	2	50	0.07
Encephalitis	4	1	3	75	0.1
Meningitis	4	4	0	0	0
Guillain-Barré syndrome	2	0	2	100	0.07
Transient gait disturbance	5	0	5	100	0.2
Confusion during fever	3	1	2	67	0.07
Miscellaneous ( <i>n</i> = 22)					
Pneumonia	12	7	5	42	0.2
Orchitis	7	6	1	14	0.03
Diabetes	3	3	0	0	0
Idiopathic thrombocytopenic purpura*					3.3

\* According to a previous study.<sup>11</sup>

vaccine that was probably responsible for the reported event was identified in 11 cases by the examinations conducted shortly after the event; streptococcal infection preceding Henoch-Schönlein purpura, influenza A infection triggering a febrile seizure, *H. influenzae* and pneumococcal infections associated with febrile seizures and otitis, herpes simplex encephalitis, *H. influenzae* and meningococcal meningitis, *H. influenzae* and *M. catarrhalis* infections concomitantly with pneumonia and otitis and 2 noninfectious factors, testicular cancer and hernia.

The serum samples of 102 vaccinees, which were analyzed later, disclosed a probable cause, or at least a contributory factor, in a further 25 cases (Table 2): pneumococcus in 7; human herpesvirus 6 in 6; *M. catarrhalis* in 3; enterovirus in 3; and *H. influenzae* in 1; 5 vaccinees had multiple infections.

In summary our clinical, serologic and epidemiologic analyses suggest that factors not related to MMR vaccination probably caused or contributed to 45% (*n* = 78) of the serious events reported. The results of this assessment and the estimated incidences are listed in Table 3.

## DISCUSSION

Large scale immunizations have sharply lowered the incidence of many vaccine-preventable diseases and their complications but raised the question of undesirable secondary effects of vaccination.<sup>31-33</sup> The risks in the community may change with time and compel changes in vaccination policy, as was the case in the US

years ago, when all residual polio cases were vaccine-induced.<sup>34</sup>

Passive notification systems are liable to the risk of underreporting, and relatively frequent but less severe events, such as febrile convulsions, are detected more reliably by active surveillance.<sup>35</sup> Clear awareness of this problem prompted the organization of an extensive campaign to motivate health care personnel and the public to report all serious events meticulously, and various efforts were made in subsequent years to maintain their interest. Because notification and sampling of paired sera were made as easy as possible, we do not regard underreporting as a major issue. Lack of a control group was realized to be another limitation but was unavoidable in an almost fully vaccinated population.

Various events were reported during the time of this study. Whether the single case of death or the chronic diseases such as asthma, epilepsy or diabetes were causally related to immunization is debatable. However, if the causality had been real, an accumulation of new cases should have occurred during the follow-up, and this did not happen. Our findings for diabetes are compatible with those of Fescharek et al.,<sup>16</sup> who found no increase in the incidence of diabetes after mumps vaccination. These estimations do not, however, exclude the possibility of a causal link in an individual case, but such a connection is highly improbable.

If one were to accept MMR vaccination as a cause of encephalitis, the facts would have to be put into per-

spective. The incidence of encephalitis is 35, 150 and 12.5 per 100 000 cases of measles,<sup>36, 37</sup> clinical mumps<sup>36, 38</sup> and rubella,<sup>36, 39</sup> respectively. We found 3 cases likely or possibly caused by MMR vaccination, 1 of these being in an immunocompromised child,<sup>28</sup> giving an incidence of 0.1 per 100 000 vaccine doses for all 3 viral components combined. This almost 2000-fold difference from natural MMR diseases, even allowing for some unreported cases, still shows that the risks of vaccination are greatly outweighed by those of wild infections. In Finland MMR vaccination has reduced by one-third all cases of childhood encephalitis.<sup>12</sup>

Aseptic meningitis develops in at least 1 per 1000 cases of clinical mumps.<sup>36, 38</sup> An association with MMR vaccine has also been identified, but virtually exclusively in recipients of the Urabe Am 9 mumps strain.<sup>40, 41</sup> In this study no case was attributable to the vaccine containing the Jeryl Lynn B strain, nor have cases of the invariably fatal subacute sclerosing panencephalitis caused by measles, with an incidence of 0.5 to 1 per 100 000 patients,<sup>36, 42</sup> occurred. Orchitis complicates 14 to 35% of mumps cases in postpubertal men, but in children it is rare.<sup>18, 43</sup> Our series disclosed only 1 suspected orchitis with possible causal relation to MMR vaccination. Approximately 50 cases a year<sup>44</sup> of congenital rubella infections have also been eliminated from Finland, not to mention all the less severe manifestations that so often required hospitalization.

No case of inflammatory bowel disease or autism was detected during this long follow-up study comprising 3 million vaccine doses. This finding is important because were there an association with MMR vaccination after such a short interval as suggested,<sup>45, 46</sup> this prospective study design would undoubtedly have disclosed at least some cases.

Some events were no doubt triggered by MMR vaccination. The estimated overall incidence of serious adverse events with a possible or unknown causal association with MMR vaccination was 3.2 per 100 000 vaccine doses or 5.3 per 100 000 vaccinees.

Revaccination caused fewer adverse events than the first vaccine dose, except for faintings, which understandably manifested among older vaccinees, and anaphylactic reactions reflecting hypersensitivity to a subsequent exposure.<sup>47, 48</sup>

Febrile seizures were the most commonly reported events. Because up to 5% of children undergo febrile convulsions before the age of 5 years,<sup>10</sup> some concurrence with vaccinations is inevitable. Concurrence was also indicated in connection with several other events, for 45% of the serious events were probably caused, at least partially, by a factor unrelated to MMR vaccine. Clearly, *post hoc non est propter hoc*, a sequence does not prove consequence.

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# Immunogenicity of a *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine when mixed with a diphtheria-tetanus-acellular pertussis-hepatitis B combination vaccine

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**Background.** Combination vaccines are urgently needed to reduce the number of injections given to young children. The aim of the study was to evaluate the safety and immunogenicity of a combination vaccine that contains diphtheria and tetanus toxoids and acellular pertussis antigens (DTaP), recombinant hepatitis B surface antigen (HepB) and *Haemophilus influenzae* type b (Hib) polysaccharide conjugated to tetanus toxoid (PRP-T).

**Methods.** Four hundred five infants were randomized equally to three groups and immunized at 2, 4 and 6 months of age with: (1) DTaP/HepB vaccine used to reconstitute lyophilized PRP-T vaccine and administered as a single injection; (2) DTaP/HepB vaccine and PRP-T vaccine administered as two separate injections; or (3) DTaP, HepB and PRP-T vaccines administered as three separate injections. Safety was closely monitored, and blood specimens were obtained to assess antibody responses to each vaccine antigen.

**Results.** All study vaccines were well-tolerated, and the rates of systemic and injection site reactions were similar between groups. After the

third dose the geometric mean antibody concentrations to Hib were significantly lower in subjects in Group 1 (1.63  $\mu\text{g/ml}$ ) compared with subjects in Groups 2 and 3 (6.26 and 6.15  $\mu\text{g/ml}$ , respectively;  $P < 0.0001$ ). Subjects with antibody concentrations  $< 1.0 \mu\text{g/ml}$  after the third dose responded well to a booster dose of Hib conjugate vaccine given at 11 to 15 months of age (41 of 44 with anti-PRP  $\geq 1.0 \mu\text{g/ml}$ ). Differences between groups for antibody responses to the other vaccine components were not clinically significant.

**Conclusions.** Infants given a combined DTaP/HepB/PRP-T vaccine experienced a significantly lower antibody response to the PRP-T component than infants given PRP-T vaccine as a separate injection. However, the immune response to a booster dose of Hib conjugate vaccine indicated the presence of immunologic memory.

## INTRODUCTION

Until 1990 children in the United States were given only six vaccine injections by 2 years of age.<sup>1</sup> During the past 10 years additional vaccines have been licensed and recommended such that children could be given up to 20 separate injections through the first 2 years of life.<sup>2,3</sup> Clearly we need new combination vaccines that will help reduce the number of injections given to children. Such vaccines would diminish the administrative costs of immunizing children, eliminate extra visits scheduled by some physicians to reduce the number of injections given at each visit, decrease the discomfort associated with multiple injections and possibly increase compliance with the overall immunization schedule.

A combination vaccine containing diphtheria and tetanus toxoids and acellular pertussis antigens (DTaP), hepatitis B (HepB) and PRP-T [*Haemophilus influenzae* type b (Hib) polysaccharide-tetanus toxoid conjugate] components has been manufactured by SmithKline Beecham Pharmaceuticals. This single vaccine would have the potential to reduce the number

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of injections given to infants by six. The purpose of this study was to evaluate the safety and immunogenicity of these vaccine components when administered to infants at 2, 4 and 6 months of age in combination as a single injection or as two or three separate injections at each visit.

## MATERIALS AND METHODS

**Subjects.** Healthy infants were recruited from two Kaiser Permanente, Southern California Region (KP-SCR) medical centers. When the infants were 6 to 12 weeks of age, informed consent to participate in the study was obtained. The study was approved by the Institutional Review Boards at KPSCR and the Harbor-UCLA Research and Education Institute. Subjects were excluded from participation if they met any of the following criteria: current rectal temperature  $\geq 38^{\circ}\text{C}$ ; immune dysfunction; major congenital defects or serious illness; neurologic or seizure disorder; receipt of any blood product or immunoglobulin preparation; previous immunization with any vaccine; or mother who is a hepatitis B carrier or HIV-positive.

**Study design.** Infants were randomized equally to three groups: Group 1, DTaP/HepB vaccine used to reconstitute lyophilized PRP-T vaccine and administered as a single injection (DTaP/HepB/PRP-T); Group 2, DTaP/HepB vaccine and PRP-T vaccine administered as two separate injections (DTaP/HepB + PRP-T); or Group 3, DTaP, HepB and PRP-T vaccines administered as three separate injections (DTaP + HepB + PRP-T). Study vaccines were administered by intramuscular injection into the anterolateral thigh(s) at ~2, 4 and 6 months of age (oral polio vaccine was given concurrently). Because the number of injections varied by study group, parents and study personnel who collected parent diary information were not blinded. However, laboratory personnel who conducted antibody assays were kept blinded.

**Vaccines.** The DTaP vaccine contained diphtheria and tetanus toxoids manufactured by the Michigan Department of Public Health and an acellular pertussis vaccine manufactured by SmithKline Biologicals (SB BIO) in Rixensart, Belgium. Each dose contained 7.5 limes flocculation units (Lf) of diphtheria toxoid, 7.5 Lf of tetanus toxoid, 25  $\mu\text{g}$  of pertussis toxoid (PT), 25  $\mu\text{g}$  of filamentous hemagglutinin (FHA), 8  $\mu\text{g}$  of pertactin (PRN) and 0.5 mg of aluminum salts (Lot 14503). The DTaP/HepB vaccine was the same as the DTaP vaccine except that it also contained 10  $\mu\text{g}$  of hepatitis B surface antigen (Lot 16509). The PRP-T vaccine was licensed and manufactured by Aventis Pasteur, Lyon, France. It was supplied as a lyophilized product and required resuspension with a diluent (either 0.4% NaCl solution or liquid DTaP/HepB vaccine). Each dose contained 10  $\mu\text{g}$  of PRP and 24  $\mu\text{g}$  of tetanus toxoid (Lot J0030). When given as a separate injection a commer-

cial lot of Engerix-B vaccine was used (Lot 1329A2). Each dose contained 10  $\mu\text{g}$  of hepatitis B surface antigen and 0.25 mg of aluminum salts. Trivalent live oral polio vaccine was obtained commercially from Lederle Laboratories, Pearl River, NY, and lot numbers were recorded at the time of vaccination.

**Safety evaluation.** At 6 h and 1, 2 and 3 days after each vaccination, the parents used standardized diary cards to record the child's rectal temperature, reactions at the injection sites and systemic symptoms. The parents mailed the completed diary cards to our office. Research personnel collected severe adverse event data from parents by telephone 1 and 3 days after each immunization and from parents and medical records at each visit.

**Antibody responses.** To evaluate responses to the study vaccines, blood specimens were obtained just before the first dose at 2 months of age, just before the third dose at 6 months of age and 1 month after the third dose at 7 months of age. Serum specimens were stored at  $-70^{\circ}\text{C}$  until assayed. The concentration of serum antibody to each vaccine antigen was assessed with standardized laboratory assay methods by personnel at the University of Rochester who were unaware of the vaccine group assignments. Antibody concentrations to PT, FHA, PRN, Hib polysaccharide (PRP) and diphtheria (D) and tetanus (T) toxoids were measured by enzyme-linked immunosorbent assay (ELISA).<sup>4</sup> Values  $<5$  ELISA units/ml (PT, FHA and PRN), 0.15  $\mu\text{g}/\text{ml}$  (PRP) and 0.10 IU/ml (D and T) were reported as undetectable. Antibody concentrations to hepatitis B surface antigen (anti-HBs) were measured by a commercial radioimmunoassay (AUSAB kit; Abbott Laboratories, North Chicago, IL) and the lower limit of detection was 10 mIU/ml.

**Booster immunization.** An interim analysis revealed that a disproportionate number of subjects in Group 1 had suboptimal antibody responses to the PRP-T component. The parents were informed of the results, and subjects (ages 11 to 15 months) in all three groups with a post-third dose antibody concentration to Hib  $<1.0$   $\mu\text{g}/\text{ml}$  were offered a booster dose with commercial PRP-T vaccine followed by a blood draw 4 to 6 weeks later. Many of the subjects had already been given a booster dose of DTwcP/HbOC vaccine (diphtheria-tetanus-whole cell pertussis and *Haemophilus b* oligosaccharide-CRM197 conjugate vaccines combined; Wyeth/Lederle Vaccines), and they were offered a blood draw 4 to 6 weeks after their booster.

**Data analyses.** The frequencies of adverse events after each dose were compared between groups by the Fisher exact test. Concentrations of antibodies to each vaccine antigen were log-transformed, and geometric mean antibody concentrations (GMC) were compared by analysis of variance. Antibody concentrations below

detectable values were assigned a value one-half of the lower limit. Statistical comparisons with  $P$  values  $\leq 0.05$  were considered significant; no adjustments were made for multiple comparisons.

## RESULTS

**Subjects.** Four hundred five infants were enrolled into the study, 135 into each of the 3 study groups. For each of the 3 groups 46 to 48% were male, 42 to 50% were Hispanic, 21 to 24% were African-American, 15 to 21% were Caucasian and 11 to 13% were of other ethnic backgrounds (not significant). The mean ages at the time of each of the 3 vaccinations were 2.1, 4.3 and 6.4 months, respectively. Fifty-four infants did not complete the study (20, 18 and 16 from Groups 1, 2 and 3, respectively) because of moving or leaving the KPSCR system (23), unsatisfactory compliance (18), parental request (10), unrelated medical problems (2) or death (1). None of the discontinuations was caused by an adverse event thought to be vaccine-related.

**Safety.** Tables 1 and 2 summarize the frequency of systemic symptoms and injection site reactions occurring within 72 h after each of the three immunizations. Overall the vaccines were well-tolerated. There were only five statistically significant differences between groups among the systemic (one) and injection site reactions (four), and these were not thought to be clinically significant. Five subjects were hospitalized or experienced a serious adverse event, including one subject who died secondary to sudden infant death syndrome 52 days after the first vaccine dose, but none was thought to be causally related to vaccination.

**Immunogenicity.** Antibody responses to all of the antigens of the vaccines are summarized in Table 3. After the second and third doses infants given the combination DTaP/HepB/PRP-T vaccine (Group 1) had significantly lower GMCs of antibody to PRP, and significantly fewer infants achieved PRP antibody concentrations  $\geq 0.15$  and  $\geq 1.0$   $\mu\text{g/ml}$  compared with infants given a separate injection of PRP-T vaccine (Groups 2 and 3). The GMCs of anti-PRP after the third

dose were 1.63, 6.26 and 6.15  $\mu\text{g/ml}$  for infants in Groups 1, 2 and 3, respectively ( $P < 0.0001$ ).

After the third dose infants given hepatitis B vaccine as a separate injection (Group 3) achieved higher anti-HBs concentrations than infants given combination vaccines; after the second dose a greater proportion of subjects in Group 3 achieved anti-HBs antibody  $\geq 10$  mIU/ml compared with subjects in Groups 1 and 2. Nevertheless after the third dose, 97 to 100% of subjects achieved seroprotective antibody concentrations in all groups.

Overall antibody responses to the three pertussis antigens and diphtheria and tetanus toxoids were comparable for infants in each of the three study groups. For pertussis a significant difference between groups was noted only for the response to pertactin after the second dose (Table 3). Although some differences were noted for the GMCs of antibody to tetanus toxoid, 100% of the infants achieved concentrations  $\geq 0.1$  IU/ml after three doses.

For 44 children who had post-third dose PRP antibody concentrations  $< 1.0$   $\mu\text{g/ml}$  (26 of 44 from Group 1), blood specimens were obtained after a booster dose of PRP-T vaccine ( $n = 12$ ) or DTwCP/HbOC vaccine ( $n = 32$ ). After the booster dose all of the infants achieved antibody concentrations to Hib  $\geq 0.15$   $\mu\text{g/ml}$ , and 41 achieved concentrations  $\geq 1.0$   $\mu\text{g/ml}$  (Table 4). The GMCs post-booster dose were 7.54 and 3.76  $\mu\text{g/ml}$  for the infants given PRP-T and DTwCP/HbOC vaccines, respectively (not significant).

## DISCUSSION

One reason for not having DTaP-based combination vaccines licensed for use in infants in the US has been the apparent interference between the DTaP and Hib conjugate components.<sup>5</sup> In our study reconstitution of PRP-T vaccine with a DTaP/HepB preparation led to a 74% reduction in the antibody response to Hib compared with separate administration of PRP-T. The proportions of infants given the combination vaccine who achieved post-third dose antibody concentrations

**TABLE 1.** Frequency of systemic adverse events within 72 h after vaccination

	No. of Injections at Each Visit	No. of Subjects	Vaccine	Temperature $\geq 101^\circ\text{F}$	Fussiness (%)	Drowsiness (%)	Poor Appetite (%)	Vomiting (%)
First dose								
Group 1	1	135	DTaP/HepB/PRP-T	0	50*	57	12	7
Group 2	2	135	DTaP/HepB + PRP-T	0.7	64*	66	19	12
Group 3	3	135	DTaP + HepB + PRP-T	2.2	58	60	16	13
Second dose								
Group 1	1	123	DTaP/HepB/PRP-T	2.4	52	42	11	7
Group 2	2	126	DTaP/HepB + PRP-T	1.6	54	41	13	10
Group 3	3	126	DTaP + HepB + PRP-T	5.6	56	42	17	7
Third dose								
Group 1	1	117	DTaP/HepB/PRP-T	1.7	46	33	12	6
Group 2	2	119	DTaP/HepB + PRP-T	1.7	40	29	9	4
Group 3	3	121	DTaP + HepB + PRP-T	6.6	43	36	17	5

\* Group 1 vs. Group 2,  $P = 0.02$ .

**TABLE 2.** Frequency of injection site adverse reactions within 72 h after vaccination

	No. of Injections at Each Visit	No. of Subjects	Vaccine	Redness (%)	Swelling (%)	Tenderness (%)
First dose						
Group 1	1	135	DTaP/HepB/PRP-T	13	13	37
Group 2	2	135	DTaP/HepB	10	15	42
			PRP-T	13	21	47
Group 3	3	135	DTaP	10	16	39
			HepB	10	17	19
			PRP-T	10	19	44
Second dose						
Group 1	1	123	DTaP/HepB/PRP-T	20*	21†	30
Group 2	2	126	DTaP/HepB	9*	9†	27
			PRP-T	11	7	26
Group 3	3	126	DTaP	17	17	33
			HepB	14	15	31
			PRP-T	15	14	32
Third dose						
Group 1	1	117	DTaP/HepB/PRP-T	14	20	29
Group 2	2	119	DTaP/HepB	15	14	19‡
			PRP-T	8	10	19§
Group 3	3	121	DTaP	15	18	33‡
			HepB	13	12	31
			PRP-T	16	14	31§

\* Group 1 vs. Group 2,  $P = 0.01$ .† Group 1 vs. Group 2,  $P = 0.02$ .‡ Group 2 vs. Group 3,  $P = 0.02$ .§ Group 2 vs. Group 3,  $P = 0.05$ .**TABLE 3.** Antibody responses to the vaccine antigens for subjects in each of the three study groups

	No. of Subjects	Vaccine	Hib			Hepatitis B*		PT	FHA	Pertactin	Diphtheria		Tetanus	
			$\mu\text{g/ml}$	% $\geq 0.15$ $\mu\text{g/ml}$	% $\geq 1.0$ $\mu\text{g/ml}$	mIU/ml	% $\geq 10$ mIU/ml				IU/ml	% $\geq 0.1$ IU/ml	IU/ml	% IU/ml
2 mo old														
Group 1	129	DTaP/HepB/PRP-T	0.11 <sup>a</sup>	24.0	1.6 <sup>b</sup>	53 (6)†	4.7	3.45	5.33 <sup>c</sup>	4.46	0.33	91.5	0.39	88.4
Group 2	131	DTaP/HepB + PRP-T	0.14 <sup>a</sup>	30.0	7.7 <sup>b</sup>	117 (10)	7.8	3.49	6.33 <sup>c</sup>	5.25	0.35	90.8	0.36	89.3
Group 3	126	DTaP + HepB + PRP-T	0.14 <sup>a</sup>	33.3	3.3 <sup>b</sup>	120 (7)	5.6	3.21	4.08 <sup>c</sup>	4.38	0.32	89.7	0.37	88.9
6 mo old														
Group 1	114	DTaP/HepB/PRP-T	0.36 <sup>d</sup>	57.0 <sup>e</sup>	31.6 <sup>f</sup>	242 (98)	86.0 <sup>g</sup>	40.6	53.6	39.3 <sup>h</sup>	0.35	94.7	1.22 <sup>i</sup>	100.0
Group 2	117	DTaP/HepB + PRP-T	1.14 <sup>d</sup>	79.5 <sup>e</sup>	61.5 <sup>f</sup>	181 (98)	83.8 <sup>g</sup>	37.4	53.4	28.9 <sup>h</sup>	0.40	98.3	1.80 <sup>i</sup>	100.0
Group 3	119	DTaP + HepB + PRP-T	0.91 <sup>d</sup>	72.0 <sup>e</sup>	55.1 <sup>f</sup>	212 (111)	94.1 <sup>g</sup>	39.6	61.8	41.3 <sup>h</sup>	0.42	96.6	1.64 <sup>i</sup>	100.0
7 mo old														
Group 1	115	DTaP/HepB/PRP-T	1.63 <sup>j</sup>	87.8 <sup>k</sup>	71.3 <sup>l</sup>	690 (112)	97.4	93.1	135	109	0.84	99.1	3.46 <sup>n</sup>	100.0
Group 2	117	DTaP/HepB + PRP-T	6.26 <sup>j</sup>	97.4 <sup>k</sup>	91.5 <sup>l</sup>	574 <sup>m</sup> (115)	98.3	87.2	121	94.4	0.79	100.0	4.42 <sup>n</sup>	100.0
Group 3	116	DTaP + HepB + PRP-T	6.15 <sup>j</sup>	98.3 <sup>k</sup>	90.4 <sup>l</sup>	910 <sup>m</sup> (116)	100.0	82.9	134	94.9	0.78	100.0	3.92	100.0

<sup>a,b</sup> Group 1 vs. Group 2 or 3,  $P \leq 0.05$ .<sup>c</sup> Group 3 vs. Group 1 or 2,  $P < 0.05$ .<sup>d,f,i,l</sup> Group 1 vs. Group 2 or 3,  $P \leq 0.0001$ .<sup>e</sup> Group 1 vs. Group 2 or 3,  $P \leq 0.001$ .<sup>i,k</sup> Group 1 vs. Group 2 or 3,  $P \leq 0.01$ .<sup>g</sup> Group 3 vs. Groups 1 and 2,  $P = 0.04$ .<sup>h</sup> Group 3 vs. Group 1 or 2,  $P < 0.05$ .<sup>m</sup> Group 2 vs. Group 3,  $P = 0.01$ .<sup>n</sup> Group 1 vs. Group 2,  $P = 0.01$ .\* Geometric mean concentrations are calculated only for those subjects with antibody to hepatitis B  $\geq 1$  mIU/ml.

† Numbers in parentheses, number of subjects.

$\geq 1.0$  and  $0.15 \mu\text{g/ml}$  were also reduced. The clinical significance of these benchmarks is unknown. Although these concentrations appeared to correlate with protection in Finnish children given unconjugated PRP vaccine,<sup>6</sup> they may not have the same relevance for conjugated vaccines in which concentrations as low as  $0.04 \mu\text{g/ml}$  may be protective.<sup>7</sup> Nevertheless antibody concentrations below  $0.15 \mu\text{g/ml}$  after the primary

series may be insufficient, particularly because the concentration decreases further before the booster immunization is given 6 to 9 months later.

Significant reductions of anti-PRP responses have been observed whether the combination vaccine includes DTaP and PRP-T vaccines alone<sup>5, 8, 9</sup> or hepatitis B and/or inactivated poliomyelitis virus (IPV) vaccines are also included.<sup>4, 10-12</sup> However, in a study by

**TABLE 4.** Antibody responses to a booster dose of Hib conjugate vaccine\*

Group	Vaccines Given for the Primary Immunization	N	Mean Age (mo) at Time of Booster	GMT ( $\mu\text{g/ml}$ )		% $\geq 0.15 \mu\text{g/ml}$		% $\geq 1.0 \mu\text{g/ml}$	
				Post-3rd dose	Post-booster	Post-3rd dose	Post-booster	Post-3rd dose	Post-booster
1	DTaP/HepB/PRP-T	26	13.0	0.20†	5.26	54	100	0	100
2	DTaP/HepB + PRP-T	8	12.5	0.28†	2.59	63	100	0	75
3	DTaP + HepB + PRP-T	10	12.9	0.52†	4.88	89	100	0	90

\* Results in this table are combined for subjects given a booster dose of PRP-T or HbOC vaccines.

† Group 1 vs. Group 3,  $P = 0.01$ ; other comparisons not significant.

Dagan et al.,<sup>13</sup> the anti-PRP response to a primary series with a combined DTaP/IPV/PRP-T vaccine (SmithKline Biologicals) was comparable with the response to a combined DTwCP/IPV/PRP-T vaccine (Aventis Pasteur, Lyon, France). After the third dose of DTaP/IPV/PRP-T vaccine, >99% of infants had anti-PRP concentrations >0.15  $\mu\text{g/ml}$ . More importantly DTaP/PRP-T combination vaccines containing a five component acellular pertussis vaccine (Aventis Pasteur, Canada) with or without IPV have demonstrated no reduction of the response to PRP-T vaccine compared with separate administration of PRP-T, and these vaccines are licensed for use in Canada.<sup>14, 15</sup> In both published reports, after the three dose primary series, >98% of infants achieved anti-PRP concentrations >0.15  $\mu\text{g/ml}$ . The five component acellular pertussis vaccine may have unique properties that do not lead to interference with PRP-T. Also whereas the Canadian vaccine is composed of antigens all from a single manufacturer, the vaccine we used had components from three different manufacturers. There may be biochemical or other factors responsible for reduced immunogenicity when antigens from different manufacturers are mixed together.

In addition to possible chemical or physical reactions between components of DTaP and PRP-T vaccines,<sup>4, 16</sup> there may be other mechanisms to help explain reduced responses to the Hib component of combination vaccines. The simultaneous administration of vaccines (either single or separate injections) that contain the same antigen, such as tetanus toxoid in diphtheria-tetanus toxoid-pertussis and PRP-T vaccines, may lead to carrier-induced epitopic suppression.<sup>17</sup> Examples of suppression include: (1) competition between B cells for specific vaccine antigens; (2) competition for the binding sites on T and B cells by free protein carrier; and (3) suppression of the binding of the conjugate to polysaccharide-specific B cells because of clonal expansion of carrier protein-specific B cells.<sup>4, 16</sup> In a study by Dagan et al.<sup>18</sup> infants given DTwCP/IPV/PRP-T vaccine concurrently with a tetravalent pneumococcal-tetanus toxoid conjugate vaccine achieved significantly lower antibody responses to PRP than those given a concurrent pneumococcal-diphtheria toxoid conjugate or a placebo at 2, 4 and 6 months of age. PRP antibody responses were inversely related to the tetanus toxoid content of

the pneumococcal-tetanus toxoid conjugate vaccine suggesting that the suppressed anti-PRP responses were the result of carrier-specific properties.

Infants in the present study with post-third dose antibody concentrations to Hib <1.0  $\mu\text{g/ml}$  were evaluated after a booster dose with PRP-T or DTwCP/HbOC vaccine. All of the subjects achieved concentrations >0.15  $\mu\text{g/ml}$  after the booster dose including infants ( $n = 16$ ) whose antibody concentrations were <0.15  $\mu\text{g/ml}$  after the primary series (data not shown). Similar responses were observed in another study of DTaP/HepB/PRP-T vaccine, in which 43 children who had low or undetectable concentrations of PRP antibody after the primary series responded well to a booster dose of HbOC vaccine (100% > 1.0  $\mu\text{g/ml}$ ).<sup>4</sup> To better assess immunologic priming, Zepp et al.<sup>10</sup> administered unconjugated PRP to children 1 year after primary immunization with DTaP/HepB/PRP-T vaccine and found anamnestic responses. Although the responses observed in our subjects given Hib conjugate vaccine at 1 year of age may have been seen in unprimed children of similar age, it appears that we did not induce immunologic tolerance to PRP with the poorly immunogenic combination vaccine.

Like others we observed interference in the response to PRP when mixed with a DTaP-based combination vaccine. Memory was likely induced with the DTaP/HepB/PRP-T vaccine, as evidenced by the booster response to Hib vaccine given 5 to 9 months later. Nevertheless the poor antibody response to the Hib component in the primary series of the combination vaccine has prevented its availability in the US.

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