

Infant Immunization and the Occurrence of Atopic Disease in Dutch and German Children: A Nested Case-Control Study

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Summary. Our goal was to assess the role of early childhood vaccination in the occurrence of respiratory symptoms and allergic sensitization in 7–8-year-old Dutch and German children. A nested case-control study was conducted among children participating in a large longitudinal study on respiratory health, to study the relationship between vaccination (bacille Calmette-Guérin (BCG), pertussis, measles/mumps, rubella, and *Haemophilus influenza* type b (Hib)) and respiratory symptoms and allergic sensitization. Parents of 510 7–8-year-old children with respiratory complaints and an equal number of randomly selected children without respiratory complaints were asked to complete a questionnaire. Blood samples were collected for specific serum IgE analysis. Vaccination status was assessed through the records of the participating Municipal Health Services. No association between vaccination against pertussis, measles, rubella, or Hib and respiratory symptoms or allergic sensitization was found. For sensitization against house dust mite, BCG vaccination resulted in an increased risk (OR, 2.28; 95% CI, 1.05–4.96). Birth order was inversely associated with allergic sensitization, but was not related to respiratory symptoms. We found an association between BCG vaccination and the subsequent risk for sensitization against house dust mite. No evidence was found for an association between vaccination and respiratory symptoms. Earlier reports of an association of birth order with atopic disease were supported by the results of the present study. **Pediatr Pulmonol.** 2004; 38:329–334. © 2004 Wiley-Liss, Inc.

Key words: respiratory symptoms; asthma; atopy; immunization; birth order; children's health.

INTRODUCTION

In recent years, the prevalence of atopic disease has increased substantially, but the causes of this increase remain unclear. As a possible explanation, Strachan¹ postulated the “hygiene hypothesis,” in which he proposed a protective effect of early childhood infections on atopic disease. Several studies using number of older siblings or daycare attendance as marker for early childhood infections are supportive of a protective effect of infections early in life.^{2,3} Vaccination, on the other hand, might promote atopic disease, either by preventing possible protective effects of natural infections or through direct immune effects, as is probably the case for bacille Calmette-Guérin (BCG), which has been thought to protect against atopy by inducing T-helper 1 (Th1)-immune responses.⁴ However, the evidence for a role of vaccination in the development of atopic disease is inconclusive.^{4–8}

Vaccination programs differ between Germany and the Netherlands, as does vaccination coverage. In Germany, poliomyelitis vaccine was administered orally until 1998, whereas in the Netherlands, poliomyelitis vaccine is coadministered with vaccination against tetanus and

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diphtheria, and normally also with pertussis. Combined measles, mumps, and rubella (MMR) vaccination is standard in the Netherlands but in Germany, although MMR vaccination is recommended, some parents prefer to vaccinate their child against measles and mumps only. Furthermore, *Haemophilus influenza* type b (Hib) was introduced in the German guidelines in 1991, just after the children participating in the present study were born. In the Netherlands Hib vaccination has been recommended since 1993.

The present study is part of a large longitudinal study on respiratory symptoms and allergies conducted in the Dutch-German borderland. Because of differences in vaccination program and vaccination coverage between Germany and the Netherlands, the data set provided the opportunity to assess the role of immunization in the development of respiratory symptoms and allergic sensitization in 7–8-year-old Dutch and German children.

METHODS

Study Population and Study Design

A nested case-control study of participants in a longitudinal study on respiratory health in children was conducted in the Dutch-German borderland, involving the Municipal Health Services of Kreis Heinsberg, Germany (250,000 inhabitants) and of the Westelijke Mijnstreek, the Netherlands (170,000 inhabitants).

In 1995, parents of all 5,052 children born between November 30, 1989–December 1, 1990 and living in the study area were invited to participate in the first survey. Parents of 3,871 children (76.6%) returned the completed questionnaire on respiratory symptoms and diagnosis. A second survey was conducted in 1997. By then, 218 children (5.6%) were lost to follow-up because they had moved out of the study area or their forwarding address was unknown. Of the remaining 3,653 children, 2,884 (78.9%) completed the 1997 questionnaire. Children with respiratory symptoms as well as controls were selected based on the answers given in the first and second surveys. All 510 children with respiratory symptoms and an equal number of randomly selected control children were included in the case-control study.

Data Collection

For both the first (1995) and second (1997) survey, data were collected in May. In autumn 1997, the case-control study was conducted. Together with an invitation for a physical examination, parental questionnaires were sent for self-completion. In the presence of one or both parents, completeness of the questionnaire was checked by an assistant of the Municipal Health Service, and blood samples were taken for immunological analysis.

Data on vaccination status and childhood infections were obtained from the Youth Health Care Department of the participating Municipal Health Services. In the Netherlands, vaccination status was registered during routine checkups of children. In Germany, vaccination status was registered during the checkup of school beginners at age 5–6 years. Data were collected on vaccination against poliomyelitis, diphtheria, tetanus, pertussis, measles, mumps, rubella, Hib, and BCG. For each individual vaccination, children were regarded as vaccinated if they had received at least one dose of the specific vaccine.

Questionnaires

The 1995 and 1997 surveys used identical questions about respiratory symptoms taken from the WHO questionnaire.⁹ To obtain data for the case-control study, an extensive questionnaire was developed, including questions about respiratory symptoms based on the ISAAC questionnaire¹⁰ and questions on indoor environment.

Definitions

Respiratory symptoms were defined as reported wheezing in the last 12 months and reported attacks of shortness of breath with wheezing in the last 12 months, or reported coughing in the morning or during the day or evening in the autumn and winter, and coughing daily for about 3 months a year, in 1995 or 1997. Control children had negative answers to all questions about respiratory symptoms in 1995 as well as in 1997.

In order to study the association between vaccination status and allergic sensitization, we additionally determined specific immunoglobulin (Ig) E in the children participating in the case-control study. Children were regarded as sensitized if at least one specific IgE tested positive, irrespective of respiratory status. Children with specific IgE against grasses or specific IgE against house dust mite (HDM) were defined separately as sensitized against grasses or against HDM, respectively. For these analyses, controls were redefined as negative for all specific IgE tested.

Blood Sampling and Determination of Immunoglobulin E

Blood samples were taken from the cubital vein of the child and, after 15 min at room temperature, were centrifuged for 10 min at 2,000g. Sera were stored at –20°C until further analysis.

Levels of specific IgE against *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria tenuis*, chicken egg, cow's milk, cat and dog epithelium, and a mix of grasses were measured using the Pharmacia CAP

System (Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden) and were regarded as positive if ≥ 0.35 kU/L.

Statistical Analysis

Univariate analyses were performed by cross-tabulation of variables and Pearson's χ^2 test. Multivariate logistic regression analysis was used to calculate adjusted risks for each individual vaccination. All regression models were adjusted for gender, birth order, country of residence, socioeconomic status, breastfeeding, exposure to environmental tobacco smoke, home dampness, pets, and childhood infections (measles, mumps, rubella, varicella, and scarlet fever). Analyses stratified according to country of residence or respiratory status were additionally performed.

All analyses were performed using SPSS PC version 10.0 (SPSS, Inc.).

RESULTS

The parents of 775 (76.0%) 7–8-year-old children returned the completed questionnaire. In 638 children, specific IgE analyses for all test allergens were performed, and for only one child was specific IgE analysis incomplete. Complete vaccination status could be retrieved for 572 of these children (89.5%). There were only marginal differences in response rates between cases and controls for the questionnaire (75.1% vs. 76.9%) and specific IgE analyses (62.5% vs. 62.7%). A somewhat larger difference was seen between cases and controls, with completed questionnaire and specific IgE analysis, for retrieval of vaccination status (87.5% vs. 91.6%).

Because of differences in national vaccination programs, vaccination status differed between German and Dutch children (Table 1). All participating German and Dutch children were vaccinated against poliomyelitis, tetanus, and diphtheria. Differing from the Netherlands, in Germany, poliomyelitis vaccination was orally administered until 1998. Because almost all children vaccinated against measles were also vaccinated against mumps,

only measles vaccination will be considered in further analyses.

No associations were seen between any of the vaccinations and respiratory symptoms or allergic sensitization in univariate analyses (Tables 2 and 3). The adjusted odds ratios (ORs) also did not show an association between vaccination and respiratory symptoms (Table 4). However, BCG vaccination increased the risk for sensitization against HDM (OR, 2.28; 95% CI, 1.05–4.96). A tendency toward an increased risk with allergic sensitization (OR, 1.74; 95% CI, 0.91–3.32) and sensitization against grasses (OR, 1.95; 95% CI, 0.89–4.31) was also seen for BCG vaccination (Table 4). Because these analyses involved both children with and children without respiratory symptoms, the same analyses were repeated stratified according to respiratory status. The association between BCG vaccination and sensitization against HDM was stronger and statistically significant only in children with respiratory symptoms (OR, 3.41; 95% CI, 1.03–11.29), but not statistically significant in children without respiratory symptoms (OR, 1.87; 95% CI, 0.45–7.82).

An inverse association between allergic sensitization and number of older siblings was found (Table 4). The same inverse association was seen in children sensitized against grasses.

DISCUSSION

It was hypothesized that vaccination may promote allergic disease by reducing clinical infections in children. In the present study, an association was found between BCG vaccination and subsequent sensitization in 7–8-year-old children. In addition, an inverse association with birth order was found for allergic sensitization but not for respiratory symptoms.

The evidence for a role of vaccination in the development of atopic disease is confusing. Although measles vaccination was reported to reduce the risk of hay fever in children with multiple older sibling contacts,⁵ for pertussis vaccination, an increased risk of atopic disease was described.¹¹ Little evidence was found for an association between Hib vaccination and atopic outcomes in East German children.¹² Other studies^{7,8,13} did not find an association between the vaccinations investigated and risk of asthma. The results of the present study are in accordance with these latter studies in showing no effect of childhood vaccination on respiratory symptoms and atopy.

Mycobacteria are potent Th1 inducers, and infection and vaccination may consequently offer protection in relation to atopy, as was reported for early BCG vaccination.⁴ Other, mostly European studies failed to show such an inverse association of BCG vaccination with atopy, defined as presence of allergen-specific IgE^{6,14} or skin prick test positivity,⁶ or using questionnaire-based

TABLE 1—Vaccination Rates for Participating German and Dutch Children¹

Vaccine	7-8-year-old children		
	All, n (%)	German, n (%)	Dutch, n (%)
BCG	75 (13.1)	74 (25.9)	1 (0.3)
Pertussis	364 (63.6)	81 (28.3)	283 (99.0)
Measles	551 (96.3)	265 (92.7)	286 (100.0)
Mumps	551 (96.3)	265 (92.7)	286 (100.0)
Rubella	438 (76.6)	152 (53.1)	286 (100.0)
Hib	247 (43.2)	247 (86.4)	0 (0)

¹All children were vaccinated against poliomyelitis, diphtheria, and tetanus.

TABLE 2—Frequencies of BCG, Pertussis, Measles, Rubella, and Hib Vaccination in Children With Respiratory Symptoms and in Sensitized Children¹

Vaccine	Respiratory symptoms			Allergic sensitization ²		
	No symptoms (n = 293)	Symptoms (n = 279)	OR (95% CI)	Not sensitized (n = 351)	Sensitized (n = 220)	OR (95% CI)
BCG	36 (12.3)	39 (14.0)	1.16 (0.71–1.89)	44 (12.5)	31 (14.1)	1.14 (0.70–1.88)
Pertussis	192 (65.5)	172 (61.6)	0.85 (0.60–1.19)	222 (63.2)	141 (64.1)	1.04 (0.73–1.47)
Measles	283 (96.6)	268 (96.1)	0.86 (0.36–2.06)	336 (95.7)	214 (97.3)	1.59 (0.61–4.17)
Rubella	226 (77.1)	212 (76.0)	0.94 (0.64–1.38)	274 (78.1)	163 (74.1)	0.80 (0.54–1.19)
Hib	122 (41.6)	125 (44.8)	1.14 (0.82–1.58)	154 (43.9)	93 (42.3)	0.94 (0.67–1.32)

¹Values are numbers (percentages).²At least one specific IgE ≥ 0.35 kU/l.

atopy definitions such as allergic disease¹⁵ and diagnosis of atopic disease.¹⁶ However, these latter studies found a protective effect in children from ethnic origin.^{15,16} Also, a Finnish study conducted among adults, using an asthma definition based on drug reimbursement registry data, found a protective influence of *Myobacterium tuberculosis* infection on asthma in women; in contrast, this infection was a risk factor for asthma in men.¹⁷

As in most previous studies, BCG vaccination was not associated with presence of respiratory symptoms at 7–8 years of age. Also, in accordance with the results of German and Swedish studies,^{15,16} we observed a (statistically nonsignificant) tendency toward lower frequency of diagnosis of asthma, hay fever, or eczema, as well as a lower frequency of antiasthma medication use among BCG-vaccinated children with asthmatic symptoms compared to non-BCG-vaccinated asthmatic children (data not shown). Factors associated with BCG vaccination, such as healthcare access, may partly explain these differences, because an indication for BCG vaccination in Western countries may selectively identify a subgroup of children with characteristics differing from the rest of the population.

In Germany, until 1998, BCG vaccination was recommended for children from regions with high tuberculosis prevalence or close contact with persons at risk. This risk-group approach resulted in a higher proportion of children from ethnic backgrounds to be BCG-vaccinated. However, most children (>95%) in our study sample were of

German or Dutch origin. Our results differ from previous results in that they tended toward a positive association between BCG vaccination and sensitization, especially against HDM. Excluding children from ethnic backgrounds from the analysis did not greatly change the results. Other factors such as low birth weight or smoking during pregnancy did not explain the results.

In a prospectively followed birth cohort of German children, transient protection against atopy was observed in BCG-vaccinated children early in life.¹⁸ However, for the proportion of positive specific IgE tests, the tendency towards lower proportion of positive tests in the BCG-vaccinated group compared to the non-BCG-vaccinated group during the first 3 years of life was reversed later in life and reached statistical significance at 5 and 7 years of age. The authors suggested that the lack of an inverse association between BCG vaccination and atopy at later age may be attributable to other environmental influences overriding an initial BCG effect.¹⁸ In our study, environmental influences associated with HDM allergen exposure and subsequent sensitization may have been associated with BCG vaccination, and may consequently have resulted in the higher risk for sensitization against HDM in BCG-vaccinated children. Ethnicity and socioeconomic status (SES) are associated with BCG vaccination in our study sample and may explain part of the positive association with HDM sensitization, through higher levels of HDM allergen exposure. For the Netherlands it was recently reported that mean Der p1 levels from mattress

TABLE 3—Frequencies of BCG, Pertussis, Measles, Rubella, and Hib Vaccination in Children Sensitized Against Grasses and in Children Sensitized Against HDM¹

Vaccine	Sensitized against grasses			Sensitized against HDM		
	No (n = 351)	Yes (n = 144)	OR (95% CI)	No (n = 351)	Yes (n = 119)	OR (95% CI)
BCG	44 (12.5)	20 (13.9)	1.13 (0.64–1.99)	44 (12.5)	20 (16.8)	1.41 (0.79–2.51)
Pertussis	222 (63.2)	92 (63.9)	1.03 (0.69–1.54)	222 (63.2)	78 (65.5)	1.11 (0.72–1.71)
Measles	336 (95.7)	142 (98.6)	3.17 (0.72–14.04)	336 (95.7)	115 (96.6)	1.28 (0.42–3.95)
Rubella	274 (78.1)	105 (72.9)	0.76 (0.48–1.18)	274 (78.1)	87 (73.1)	0.76 (0.47–1.23)
Hib	154 (43.9)	58 (40.3)	0.86 (0.58–1.28)	154 (43.9)	56 (47.1)	1.14 (0.75–1.73)

¹Values are numbers (percentages).

TABLE 4—Odds Ratios and 95% Confidence Intervals for Association Between Risk Factors and Atopic Disease¹

	Respiratory symptoms		Allergic sensitization ²		Sensitized against grasses		Sensitized against HDM	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Gender (male vs. female)	1.68	1.13–2.49 ³	2.68	1.76–4.09 ³	3.44	2.05–5.76 ³	2.90	1.69–4.96 ³
Birth order ⁴								
Only younger siblings	1.06	0.54–2.08	0.57	0.29–1.13	0.52	0.24–1.12	0.74	0.30–1.83
1 older sibling	0.91	0.47–1.76	0.47	0.24–0.92 ³	0.44	0.20–0.95 ³	0.75	0.31–1.82
2 older siblings	1.63	0.74–3.60	0.40	0.18–0.91 ³	0.40	0.16–1.02	0.69	0.24–1.97
>2 older siblings	0.95	0.30–3.02	0.33	0.09–1.15	0.20	0.04–1.13	0.29	0.05–1.71
BCG ⁴	1.17	0.63–2.19	1.74	0.91–3.32	1.95	0.89–4.31	2.28	1.05–4.96 ³
Pertussis ⁴	0.83	0.45–1.52	0.89	0.47–1.70	0.84	0.38–1.84	1.02	0.46–2.25
Measles ⁴	0.93	0.30–2.90	1.51	0.43–5.35	2.85	0.45–18.08	1.93	0.38–9.95
Rubella ⁴	1.17	0.65–2.10	0.85	0.46–1.57	0.75	0.36–1.56	0.89	0.41–1.92
Hib ⁴	1.39	0.60–3.19	0.74	0.30–1.79	0.55	0.19–1.58	1.14	0.33–3.89

¹Adjusted for breastfeeding, exposure to environmental tobacco smoke, home dampness, pets, country of residence, socioeconomic status, and childhood infections (measles, mumps, rubella, varicella, and scarlet fever).

²At least one specific IgE ≥ 0.35 kU/l.

³ $P \leq 0.05$.

⁴Reference category: risk factor not present.

dust were three times higher among Moroccan immigrant children compared to Dutch children or Turkish immigrant children, and consequently sensitization was also most frequent in Moroccan children.¹⁹ Besides ethnicity or SES, other factors not accounted for in the present study may likewise be associated with factors (such as way of heating or presence of insulation measures) contributing to higher indoor HDM allergen levels.

The argument that vaccination presents a risk because it reduces natural infection is not a likely explanation for our finding. Protective effects of *Mycobacterium tuberculosis* infection in childhood on asthma and atopy were reported,¹⁷ but tuberculosis incidence in Germany is low, and none of the German subjects in the present study were reported to have a tuberculosis infection.

The inverse association of birth order with atopic manifestations seen in the present study was repeatedly found in many but not all studies.^{11,20–22} It is at present uncertain which factors cause this birth-order effect. Our findings are in accordance with the “hygiene hypothesis,”²⁰ but alternative explanations focusing on in utero programming and endocrine effects²³ have also been proposed.

After adjustment for various childhood infections (measles, mumps, rubella, chickenpox, and scarlet fever), the association with birth order remained, indicating that other infections than those adjusted for were responsible for this birth-order effect. However, sibling order may represent differences in timing or dose of exposure, as suggested for measles infection in children from a British national birth cohort, in whom contact with older siblings seemed to be a prerequisite for a protective effect of measles.⁵ In addition, birth order may represent factors other than exposure to infection, such as changes in in utero environment and in some of the mother's lifestyle

characteristics likely to change with parity.²³ In our study sample, children from atopic mothers (self-reported asthma, hay fever, or eczema ever), especially children from asthmatic mothers, more often had no siblings and less often older siblings than children from nonatopic or nonasthmatic mothers. Furthermore, paternal asthma was not associated with number of siblings. Previously it was also observed that atopic mothers had a lower number of offspring, and since maternal atopy is a strong risk factor for childhood atopy, this could partly explain the association between atopy in the child and birth order.²⁴

Vaccinated and unvaccinated children may differ in other respects than those accounted for in the regression models. For instance, atopic parents may be more reluctant to vaccinate their children than nonatopic parents. In our data, we found no evidence of selective avoidance of vaccination of their child by parents with self-reported asthma.

A limitation of the present study design is that children were selected based on answers to questions about respiratory symptoms in previous surveys, and sensitization was determined afterwards in the case-control study. Analyses stratified by respiratory status showed no evidence of an association between atopic disease and vaccination in children with respiratory complaints or in children without complaints, and the elevated risk for HDM-sensitization with BCG vaccination was seen only in children with respiratory symptoms. Also, the case group consisted of children with wheezing or coughing symptoms. However, most children (75.0%) had ever had wheezing, and 43.7% reported suffering from wheezing in the past 12 months. Narrowing our definition of respiratory symptoms down to children with only asthmatic symptoms, recent wheezing at age 7–8 years, or asthmatic symptoms already present at age 5 did not change the

results. Failure to find a consistent relationship between childhood asthma and respiratory infections may lie in the lack of a clear phenotypic description of asthma.²⁵ If the same applies to vaccination, our case definition may have diluted possible vaccination effects. However, because of the low number of BCG-vaccinated children, the power to detect an association between BCG vaccination and sensitization was low. Consequently the association must be strong to reach statistical significance.

Although we acknowledge the potential problem of misclassification of disease outcome when relying on parental reports, we used reported respiratory symptoms instead of asthma diagnosis because of the large differences in prevalence rates of diagnosis between German and Dutch children.²⁶

A large advantage of the present study was that exposure (vaccination) and outcome (atopic disease) were independently assessed, so information bias is not likely to have greatly influenced our data. Also, considering only children born in 1 year excluded age as an important confounder.

In conclusion, the results provide no evidence for an association between vaccination and respiratory symptoms. However, positive associations were found between early childhood BCG vaccination and sensitization to house dust mite. Further studies to elucidate the mechanisms underlying the effects of BCG vaccination on atopic disease are needed, as the possible role of mycobacteria in the prevention and treatment of atopic disease is currently being discussed. Earlier reports of an association of birth order with atopic disease were supported by the results of the present study.

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