



Research Paper

Vaccination and Allergic Sensitization in Early Childhood – The ALADDIN Birth Cohort

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ABSTRACT

Background: The increasing incidence of allergic diseases highlights the importance of finding underlying mechanisms. Early vaccination has been suggested as one influential factor. However, it is difficult to find a study group with a large variation between subjects concerning compliance to the official vaccination program. The anthroposophic lifestyle is of interest in this context. Moreover, cohort studies show that children of families with this lifestyle run a lower risk of allergic sensitization and allergy-related disease.

Methods: From the prospective birth cohort ALADDIN we included one group from the anthroposophic community, with restrictive attitudes concerning vaccinations, and two other groups of age-matched children with more conventional parental lifestyles. In all, 466 children were followed from birth to five years of age. Detailed vaccination data and blood samples were collected at six months, one, two, and five years. Information was also obtained on risk factors for allergy. The outcome variable, allergic sensitization was defined as allergen-specific serum IgE levels ≥ 0.35 kU_A/L.

Findings: In a logistic regression model adjusted for socio-demographics and established allergy risk factors, vaccination at later age or having a lower number of injections or vaccines were associated with low OR for allergic sensitization during the first year of life. However, after adjustment for anthroposophic lifestyle, no statistically significant associations remained. The adjusted OR for sensitization at five years of age in children not receiving any vaccinations ($n = 54$) was 0.98 [95% CI 0.38–2.57].

Interpretation: We found no support for an association between early childhood vaccination and subsequent allergic sensitization. Our findings do not support scepticism towards early childhood vaccination motivated by allergy risk.

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1. Introduction

The increasing incidence of allergic diseases over the last decades highlights the importance of finding etiological factors, especially those having changed over time. One explanation commonly referred to is the hygiene hypothesis. It proposes that the rising incidence of IgE-mediated allergy diseases in industrialized countries is a

Abbreviations: ALADDIN, Assessment of Lifestyle and Allergic Diseases During Infancy; DPPT, Diphtheria, Pertussis, Polio, Tetanus; DTP, Diphtheria, Tetanus, Pertussis; DTaP, Diphtheria, Tetanus, acellular Pertussis; Pentavac™, Diphtheria, Tetanus, Pertussis, Polio, *Haemophilus influenzae* type b; Tetravac™, Diphtheria, Tetanus, Pertussis, Polio; MCHC, Maternal and Child Healthcare Centres; MMR, Measles, Mumps, Rubella; NIP, the National Swedish Immunization Program; OR, odds ratio.

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consequence of increasingly sanitary living conditions, leading to less exposure of the child's developing immune system to environmental microbes and parasites [1]. This decline in pathogen exposure is assumed to disrupt the balance in T-cell immune responses, promoting Th2 responses that drive the development of allergic diseases in young individuals. It has been hypothesized, as a consequence, that vaccination in childhood may influence the development of allergy due to prevention of the clinical disease in question [2]. In addition, the vaccine per se might mediate a non-desired shift in the immune system, for example due to additives like adjuvants and preservatives which come with each dose of most vaccines [3].

Some parents are sceptical of the national vaccination program, which is a complementary reason to seek more reliable data from studies with appropriate designs [4]. One methodological problem is that it is difficult to find a study group in a Western country where there is a large enough variation between subjects concerning compliance to the

Research in Context

Evidence Before This Study

Previous studies on the association between vaccination and subsequent risk of allergic disease are inconclusive [2,7,12,13]. Data have mostly been based on retrospective or cross-sectional studies and are thus prone to bias from reverse causation [13]. Longitudinal data indicate either no association or an inverse association with allergic disease, but these reports are sparse and reference groups vary [14,15]. Studies on DTP (Diphtheria, Tetanus, Pertussis) vaccination are inconsistent, with some having found increased risk of asthma and allergy in pertussis-vaccinated children [12,16], while other studies found no relation [17,18,19]. A more comprehensive vaccination program in the first year of life in children with established atopic dermatitis was not associated with an increased risk of more severe eczema or allergic sensitization at two years of age [2]. A longitudinal study found no association between common childhood immunizations and risk of asthma or atopic disease in middle age [20].

The immune system of a child matures gradually and is considered to be most dynamic early in life [21]. In a large retrospective cohort, recruited at vaccination centres, postponing the first dose of DTaP (Diphtheria, Tetanus, acellular Pertussis) vaccination was associated with less prevalent eczema, but not with food allergy or atopic sensitization [22]. In a retrospective birth cohort, not covering sensitization, no relation was found between the age at first vaccination for either DPPT (Diphtheria, Pertussis, Polio, Tetanus) or MMR and the risk of asthma or eczema [23]. Thus, from a theoretical perspective and based on the non-conclusive findings of previous studies, it is motivated to include age at first vaccination in the analyses when studying any influence of vaccination upon the development of allergy-related outcomes. Immune-stimulation becomes more diverse from polyvalent than monovalent vaccines and it is of interest to study whether there is an association between the total number of vaccine doses against infectious diseases given and subsequent IgE development. Moreover, both mono- and polyvalent vaccines (with the exception of MMR) have a fairly similar content of additives such as preservatives and immune-stimulants, usually aluminum salts [3]. An overview of human and animal studies concludes that there are no indications of harmful effects of additives in this respect, with the exception of rare hypersensitivity reactions [24]. However, in animal models aluminum salts may induce a dysregulation, leading to allergic diseases, especially in genetically susceptible individuals [25]. To summarize, it is motivated to include analyses of associations between the number of injections and the total sum of vaccines received in relation to the development of allergic sensitization.

Concerns have been raised that MMR vaccination may have contributed to the increase in prevalence of allergic diseases, either by a negative effect of the vaccine per se, or by protecting from the relevant infections, especially measles [3,7]. Notably, the design and reporting of safety outcomes in MMR vaccine studies have been criticized [11], and the methodological quality of many studies made it difficult to generalize their results [11].

Added Value of This Study

In this study of infants followed from birth up to five years of age – including an anthroposophic group with its associated low incidence of allergy and parental restrictiveness against vaccination – we found no evidence of a relation between four child vaccination

characteristics (age at first vaccination; total number of vaccinations; total number of vaccines; MMR vaccination) and allergic sensitization.

The children from the non-anthroposophic families were representative of Swedish children both regarding sensitization and vaccination patterns, which allows for generalization of our findings. In contrast to previous studies [20,26,27], the number of children with an individualized vaccination program was high and the number of children unvaccinated up to five years of age was uniquely high.

Implications of All the Available Evidence

Our findings do not support scepticism towards vaccination motivated by allergy risk. However, the co-variation of exposures related to anthroposophic lifestyle, including delayed or avoided vaccination, implies a limited statistical power to disentangle explanations of the low sensitization to allergens in children with this lifestyle.

official vaccination program. The anthroposophic lifestyle is of interest in this context. Firstly, cross-sectional studies have shown that children of families with this lifestyle run a lower risk of allergic sensitization and allergy-related disease [5,6,7]. Secondly, the families are restrictive concerning vaccinations, which is expressed in both a lower degree of vaccinations and a later start of the vaccination program [8].

The anthroposophic lifestyle is characterized by, for instance, a preference for home deliveries, a long period of breastfeeding, a diet of organic/biodynamic products emphasizing vegetarian components, and a restrictive use of antibiotics and antipyretics [8]. Furthermore, the importance of a holistic view of life is emphasized, including spiritual values [9]. Thus, any studies concerning factors influencing allergy development in this group need to include comparison groups with a more “conventional” lifestyle.

The aim of this study was to investigate if a restrictive vaccination program within a prospectively assessed population was associated with risk of allergic sensitization. Based on both theoretical and empirical perspectives, age at first vaccination, number of vaccinations, and number of vaccines were selected as primary exposure variables. Since MMR (Measles, Mumps, Rubella) vaccination has awakened particular concerns in relation to allergy [10,11], we focused some analyses on this exposure variable.

2. Methods

2.1. Study Design and Participants

This study is based on ALADDIN, a prospective birth cohort study that focuses on the impact of lifestyle and environmental factors during pregnancy and childhood on the development of allergic disease [9]. In all, 312 families were recruited at anthroposophic maternal and child healthcare centres (MCHC) and 240 families at conventional MCHCs, all in the Stockholm area, from September 2004 until February 2011. Families with infants born before gestational week 35 were excluded, as well as those with miscarriages. Based upon the parents' choice of antenatal clinic and their responses to three questions when their child was 2 months old they were grouped as “Anthroposophic”, “Partly anthroposophic” or “Non-anthroposophic”. The questions were: 1) “What kind of pre-school/school will your newborn child probably go to?” 2) “Has any of the parents, no matter which type of school you have planned for your child, an anthroposophic view of life?” and 3) “Is the family's everyday life influenced by an anthroposophic view of life?” Families answering

Table 1

The national Swedish immunization program (NIP) offered to children up to five years of age.^b

Age	3 months	5 months	12 months	18 months	5 years
Vaccine against					
Diphtheria	Dose 1	Dose 2	Dose 3		Dose 4
Tetanus					
Pertussis					
Polio					
Hib type B ^a	Dose 1	Dose 2	Dose 3		
Pneumococcal disease	Dose 1	Dose 2	Dose 3		
Measles				Dose 1	
Mumps					
Rubella					

^a Since 2013, a hexavalent vaccine including hepatitis B has been used in Stockholm county.

^b In 2009, vaccination against invasive pneumococcal disease was introduced into the NIP.

“anthroposophic school” to question 1 and “yes” to question 2 and 3 and also attending anthroposophic antenatal clinics were defined as “Anthroposophic”. Families answering conventional or any other non-anthroposophic type of school to question 1, “no” to question 2 and 3 and going to conventional antenatal clinic were defined as “Non-anthroposophic”. Any other combination of answers was defined as “Partly anthroposophic”. Furthermore, there had to be data on lifestyle, a medical record on children’s vaccinations and results from at least one blood sample obtained between six months and five years of age analyzed for allergen-specific IgE (see below). From a total of 552 recruited families, 32 families did not report their lifestyle. Thus, 520 entered the study protocol, of which 466 children fulfilled the inclusion criteria, 363 at six months of age, 372 at one year, 396 at two years, and 339 at five years of age.

2.2. Procedures

At child ages of six months, one, two, and five years, the parents filled in questionnaires and were interviewed concerning data related to allergy. Information on vaccination history was collected from medical records at child healthcare centres. If not available at the centres, they were collected from a central county vaccination register. A parental questionnaire concerning demography, as well as health and lifestyle-related aspects, was filled in when the child was two months old [9]. Data for the adjustment variables (see below) were collected from this questionnaire.

Blood samples were obtained from children at six months, one, two, and five years of age, as well as from the parents at inclusion in the study. Mean child ages at collection of blood samples for assessment of allergic sensitization at 6 months, one, two, and five years were 6.2, 12.3, 24.4 and 61.9 months. Samples were collected in heparin tubes and plasma was stored at -20°C . Plasma from the children at six months, one, and two years of age was analyzed using ImmunoCAP

Table 2

Demographic and allergy-related data in the different lifestyle groups (n = 466).

	Anthro (n = 99; 21.2%)		Partly anthro (n = 204; 43.8%)		Non-anthro (n = 163; 35.0%)		p*
	n	%	n	%	n	%	
Gender (boy)	51/99	(51.5)	100/204	(49.0)	77/163	(47.2)	0.80
Mother university-educated	50/98	(51.0)	104/202	(51.5)	74/161	(46.0)	0.55
Parental sensitization**	65/99	(65.7)	117/192	(60.9)	96/160	(60.0)	0.65
Older siblings***	76/98	(77.6)	121/199	(60.8)	100/159	(62.9)	0.012
Family living on a farm with animals	11/98	(11.2)	14/202	(6.9)	11/160	(6.9)	0.66
Mother smoking during pregnancy	5/98	(5.1)	11/201	(5.5)	9/161	(5.6)	1.00
Breastfed fully at 6 months	40/99	(40.4)	68/201	(33.8)	19/162	(11.7)	<0.001

Anthro = anthroposophic, partly anthro = partly anthroposophic, non-anthro = non-anthroposophic.

* Fisher’s exact test was used for p values.

** Parental sensitization = positive Phadiatop™ = allergen-specific IgE levels $\geq 0.35\text{ kU}_A/\text{L}$.

*** More than one sibling.

(Thermo Fisher Scientific, Uppsala, Sweden) for IgE against cow’s milk, hen’s egg, peanut, cat, dog, birch, and timothy, and at age five years, using a food mix (fx5) (Thermo Fisher Scientific) and Phadiatop™ (adding codfish, wheat flour, soy bean, *Cladosporium*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, horse, and mugwort). Parental sensitization was defined using ImmunoCAP Phadiatop™ (Thermo Fisher Scientific) for IgE antibodies against 11 inhalant allergens.

The regional research ethics committee in Stockholm approved the study. Written informed consent was obtained from all families.

2.3. The Swedish Vaccination Program for Children

The national immunization program (NIP) includes vaccination against nine different infectious diseases up to five years of age (Table 1). There is a well-established child healthcare system in Sweden which has had 96–98% coverage of the vaccinations recommended in the NIP in infancy for many decades [28]. Children at risk are also offered Bacillus Calmette-Guérin (BCG) vaccine against tuberculosis and vaccine against hepatitis B. In the county of Stockholm, a hexavalent vaccine including hepatitis B has been used since 2013.

2.4. Exposure and Outcome Variables

The following exposure variables were selected:

- [1] Age at first vaccination (regardless of vaccine type). For the logistic regression analyses, age at vaccination was defined as the interval before the actual age when the blood sample was obtained. There were also four “no vaccination at the age” variables, at six months and at one, two, and five years of age.
- [2] Total sum of vaccine injections (Sum injections) at six months and at one, two, and five years of age.
- [3] Total sum of vaccines (Sum valences) at six months and at one, two, and five years of age. For example, a child given Pentavac™ (five valences) three times, MMR (three valences) once and Tetravac™ (four valences) once would be categorized as: sum injections = five; sum valences = 22.
- [4] MMR, first dose at age 18 months (only measured at ages two and five years).

The outcome variable, allergic sensitization, was defined as allergen-specific IgE levels $\geq 0.35\text{ kU}_A/\text{L}$. Data are presented in relation to any allergen (AnySens), to food allergens (FoodSens) and to airborne allergens (AirSens), respectively.

Adjustment variables used in the logistic regression analyses were:

- [1] Socio-demographic variables: Sex of the child; maternal education.
- [2] Established allergy risk variables [7,8,29]: Parental sensitization; Number of siblings or other children living with the family;

Table 3

Cumulative vaccination history in children from six months up to five years of age in relation to lifestyle. Age at first vaccination median (min-max). Sum injections, and Sum valences mean (min-max).

	Anthroposophic	Partly anthroposophic	Non-anthroposophic	p*
Total number of children	99	204	163	
Age at 1st vaccination months	18.5 (2.5-65.5)	7.7 (1.6-85.9)	3.3 (2.3-62.6)	<0.001
Any vaccination n (%)				
6 months	5 (5.1)	62 (30.4)	136 (83.4)	<0.001
1 year	17 (17.2)	98 (48.0)	155 (95.1)	<0.001
2 years	41 (41.4)	131 (64.2)	160 (98.2)	<0.001
5 years	58 (58.6)	153 (75.0)	161 (98.8)	<0.001
MMR				
2 years	3 (3.0)	43 (21.1)	128 (78.5)	<0.001
5 years	9 (9.1)	65 (31.9)	138 (84.7)	<0.001
Measles, Mumps, Rubella				
Sum injections				
6 months	0 (0-4)	0 (0-5)	2 (0-7)	<0.001
Number				
1 year	0 (0-4)	0.5 (0-7)	3 (0-7)	<0.001
2 years	0 (0-7)	2 (0-8)	4 (0-10)	<0.001
5 years	1.5 (0-8)	3 (0-10)	5 (0-14)	<0.001
Sum valences				
6 months	0 (0-14)	0 (0-15)	10 (0-22)	<0.001
Number				
1 year	0 (0-14)	0.5 (0-22)	12 (0-22)	<0.001
2 years	0 (0-21)	8 (0-25)	18 (0-32)	<0.001
5 years	3 (0-25)	12 (0-25)	21 (0-32)	<0.001

* The Kruskal-Wallis test was used to compare the median age at first vaccination and the Fisher's exact test for all other variables.

Family living on a farm with animals; Mother smoking during pregnancy; Exclusive breastfeeding at age six months [8,9].

[3] Lifestyle variables: Anthroposophic; Partly anthroposophic; Non-anthroposophic [9].

2.5. Statistical Analyses

SPSS Statistics 21.0 (IBM Software, IL, USA) was used for the statistical analyses. Comparisons between the lifestyle groups with regard to background characteristics and vaccination history were made with Fisher's exact test. The Kruskal-Wallis test was used to compare the median age at first vaccination.

Associations between the exposure variables and each of the outcome variables (allergic sensitization at age 6 months and at one, two, and five years of age), were assessed in logistic regression analyses one at a time. Three models were used for each of the outcome variables in these analyses:

Model 1) unadjusted – association between outcome and one exposure variable.

Model 2) adjusted for the socio-demographic variables and established allergy risk variables described above.

Model 3) adjusted like model 2, as well as for anthroposophic lifestyle.

Odds ratios (Or's) and 95% confidence intervals (CI) were calculated. p values < 0.05 were considered significant.

2.6. Role of the Funding Source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

Data on sociodemographic, allergy risk and anthroposophic lifestyle-related variables are presented in Table 2.

Presence of older siblings and having been breastfed at six months differed between the lifestyle groups, with highest numbers in the anthroposophic group. There were no significant differences in sensitization between parents with different lifestyles; anthroposophic 65.9%, partly anthroposophic 60.9% and non-anthroposophic 60.0%.

Comparisons of vaccination history between lifestyle groups showed marked differences (Table 3). The proportion of unvaccinated children in the three lifestyle groups – anthroposophic, partly anthroposophic and non-anthroposophic – were, at age six months 94.9%; 69.6%; 16.6%, at one year of age, 82.8%; 52.0%; 4.9%, at two years of age 58.6%; 35.8; 1.8%, and at age five years of age 41.4%; 25.0%; 1.2%.

All sensitization measures (“any”, “food”, “air”) differed between the lifestyle groups and sensitization to allergens was consistently most

Table 4

Cumulative sensitization* in children at different ages up to five years in relation to lifestyle.

Total number of children	Anthroposophic		Partly anthroposophic		Non-anthroposophic		p**
	99		204		163		
	Sensitized/all available samples	Percent	Sensitized/all available samples	Percent	Sensitized/all available samples	Percent	
AnySens	6 months	2/70 (2.9)	14/156 (9.0)	25/137 (18.2)	0.002		
	1 year	3/70 (4.3)	22/163 (13.5)	37/139 (26.6)	<0.001		
	2 years	13/85 (15.3)	22/164 (13.4)	35/147 (23.8)	0.052		
	5 years	23/74 (31.1)	40/152 (26.3)	51/103 (45.1)	0.006		
FoodSens	6 months	1/74 (1.4)	12/168 (7.1)	22/141 (15.6)	0.001		
	1 year	2/74 (2.7)	20/166 (12.0)	37/145 (25.5)	<0.001		
	2 years	8/85 (9.4)	19/167 (11.4)	27/148 (18.2)	0.106		
	5 years	13/74 (17.6)	22/152 (14.5)	39/113 (34.5)	<0.001		
AirSens	6 months	1/70 (1.4)	3/155 (1.9)	7/137 (5.1)	0.287		
	1 year	1/69 (1.4)	4/162 (2.5)	8/137 (5.8)	0.241		
	2 years	5/85 (5.9)	8/164 (4.9)	17/147 (11.6)	0.076		
	5 years	14/74 (18.9)	28/152 (18.4)	28/112 (25.0)	0.400		

* Allergen-specific IgE ≥ 0.35 kU_A/L for any allergen (AnySens), for food allergens (FoodSens), for airborne allergens (AirSens).

** Fisher's exact test was used for p values.

Table 5

Association between vaccination data and allergic sensitization at six months, one, two, and five years, logistic regression.

Exposure		AnySens 6 months					AnySens 1 year				
		AnySens		Model 1	Model 2	Model 3	AnySens		Model 1	Model 2	Model 3
		N	% AnySens	OR (95% CI)	OR (95% CI)	OR (95% CI)	N	% AnySens	OR (95% CI)	OR (95% CI)	OR (95% CI)
[1] Age at first vaccination*	0-5.99	192	16	Reference			198	22	Reference		
months	Unvaccinated before 6 m	171	6	0.37 (0.18-0.77)	0.41 (0.18-0.91)	1.21 (0.39-3.79)	41	17	0.72 (0.30-1.73)	0.79 (0.31-1.99)	2.28 (0.74-7.09)
	6-11.99						133	8	0.32 (0.16-0.64)	0.37 (0.17-0.81)	1.51 (0.48-4.72)
	Unvaccinated before 1 yr.						132	8	0.32 (0.16-0.65)	0.33 (0.15-0.74)	0.88 (0.31-2.47)
[2] Sum inj.**	0	171	6	0.31 (0.15-0.66)	0.33 (0.14-0.76)	0.87 (0.29-2.68)	19	5	0.20 (0.03-1.51)	0.19 (0.02-1.52)	0.37 (0.04-3.12)
Number	1	43	9	0.47 (0.15-1.42)	0.42 (0.12-1.52)	0.48 (0.13-1.77)	167	22	Reference		
	2-4	144	18	Reference			54	24	1.11 (0.54-2.30)	0.85 (0.37-1.92)	0.65 (0.28-1.52)
	5-7	5	0	-	-	-	132	8	0.31 (0.15-0.63)	0.33 (0.15-0.72)	0.90 (0.31-2.59)
[3] Sum valence**	0	171	6	0.27 (0.13-0.57)	0.28 (0.11-0.67)	0.75 (0.23-2.42)	33	6	0.22 (0.05-0.96)	0.23 (0.05-1.02)	0.50 (0.10-2.41)
Number	1-9	74	8	0.34 (0.13-0.88)	0.33 (0.11-0.95)	0.42 (0.14-1.24)	194	23	Reference		
	10-18	117	21	Reference			13	40	2.13 (0.66-6.84)	0.92 (0.22-3.81)	0.79 (0.19-3.29)
	19-22	1	0	-	-	-					
Exposure		AnySens 2 years					AnySens 5 years				
		AnySens		Model 1	Model 2	Model 3	AnySens		Model 1	Model 2	Model 3
		N	% AnySens	OR (95% CI)	OR (95% CI)	OR (95% CI)	N	% AnySens	OR (95% CI)	OR (95% CI)	OR (95% CI)
[1] Age at first vaccination*	0-5.99	205	22	Reference			171	39	Reference		
6-24 months	6-11.99	41	12	0.51 (0.19-1.37)	0.65 (0.23-1.80)	1.08 (0.34-3.50)	31	36	0.88 (0.39-1.94)	0.96 (0.41-2.23)	1.61 (0.62-4.17)
	12-23.99	52	17	0.77 (0.35-1.69)	0.94 (0.41-2.14)	1.61 (0.53-4.88)	47	21	0.43 (0.20-0.92)	0.50 (0.23-1.12)	0.97 (0.36-2.62)
	Unvaccinated before 2 yrs.	98	12	0.51 (0.26-1.02)	0.56 (0.26-1.22)	0.90 (0.31-2.60)	36	39	1.01 (0.48-2.12)	1.31 (0.56-3.04)	2.50 (0.89-7.05)
[1] Age at first vaccination*	24-59.99						54	24	0.50 (0.25-1.01)	0.52 (0.24-1.14)	0.98 (0.38-2.57)
24-60 months	Unvaccinated before 5 yrs.						57	25	0.56 (0.28-1.10)	0.58 (0.27-1.27)	0.96 (0.40-2.29)
[2] Sum inj.**	0	95	11	0.44 (0.21-0.93)	0.49 (0.22-1.11)	0.73 (0.26-2.07)	89	32	0.79 (0.45-1.36)	0.88 (0.49-1.61)	1.44 (0.70-2.94)
Number of inj.	1-3	106	17	0.76 (0.41-1.42)	0.88 (0.45-1.70)	1.30 (0.56-3.02)	160	37	Reference		
	4-7	180	21	Reference			32	41	1.17 (0.54-2.54)	1.01 (0.43-2.46)	1.06 (0.44-2.52)
	8-14	15	27	1.36 (0.41-4.51)	1.04 (0.27-4.10)	1.08 (0.27-4.31)	57	25	0.55 (0.27-1.10)	0.58 (0.27-1.26)	0.92 (0.36-2.36)
[3] Sum valence**	0	95	11	0.43 (0.20-0.91)	0.47 (0.21-1.08)	0.70 (0.24-2.07)	112	28	0.64 (0.37-1.10)	0.72 (0.40-1.31)	1.15 (0.53-2.48)
Number of inj.	1-17	121	17	0.76 (0.42-1.39)	0.85 (0.44-1.62)	1.29 (0.55-3.01)	131	38	Reference		
	18-22	162	22	Reference			38	53	1.86 (0.90-3.85)	1.94 (0.89-4.23)	2.06 (0.93-4.59)
	23-32	18	22	1.04 (0.32-3.35)	0.76 (0.20-2.96)	0.76 (0.20-2.95)	155	30	0.66 (0.42-1.05)	0.77 (0.47-1.28)	1.40 (0.72-2.74)
[4] MMR	No	223	14	0.60 (0.35-1.00)	0.60 (0.34-1.07)	0.85 (0.37-1.91)	169	39	Reference		
	Yes	173	22	Reference							

[1-4] Exposure variables, see [Statistical Analyses](#).

Model 1 unadjusted.

Model 2 adjusted for risk factors: sex of the child (male), parental sensitization, mother's education, mother smoking during pregnancy, number of siblings or other children living with the family, family living on a farm with animals, and exclusive breast-feeding at age 6 months.

Model 3 adjusted like model 2, as well as for lifestyle (anthroposophic, partly anthroposophic, non-anthroposophic).

* Age at first vaccination was calculated as the number of months from the birth date to the date of the first vaccination, where a month was defined as 30 calendar days.

** Example: Pentavac™ (valence 5) given three times, MMR (valence 3) once and Tetravac™ (valence 4) once: sum injections = 5, sum valence = 22.

prevalent in the non-anthroposophic group and least prevalent in the anthroposophic group (Table 4).

Table 5 shows the association between the vaccination variables and allergic sensitization at six months and at one, two, and five years of age (for MMR at age two and five years). The range set as reference in Table 5 is the range that includes the exposure corresponding to those that follow our national immunization program (NIP). For example, the reference for age at first vaccination corresponds to the recommendation that children should start their vaccination program before they are six months old. When unadjusted (model 1), restricted vaccination (later age, lower sums of injections and of vaccines) was associated with a low OR for allergic sensitization, primarily during the first year of life, with a similar association when adjusting for risk factors for allergic sensitization (model 2). However, after adjustment for anthroposophic lifestyle (model 3), no statistically significant associations remained. Avoiding MMR vaccination did not affect the risk of allergic sensitization. Performing the same calculations separately for children sensitized to food and air allergens, respectively, showed similar results (data not shown).

4. Discussion

In this study of a group of anthroposophic children followed up to five years of age – with previously known lower incidence of allergy and restrictiveness against vaccination as compared to the general population [9] – and two comparison groups with more conventional lifestyles, we found no evidence of a relation between vaccination and allergic sensitization after adjusting for gender, socio-economic status, some established allergy risk factors, and anthroposophic lifestyle.

Most of the recent epidemiological studies on vaccination and atopy conclude that the main current vaccines do not cause allergic diseases [14,15,20,26], which is in line with our findings. We also conclude, like McKeever et al. [23], that the total number of vaccinations given is not associated with allergic disease. Furthermore, we compared the incidence of allergic sensitization at two and five years of age in those who had or had not received MMR vaccination, but found no association to allergic sensitization. These findings are in agreement with results from some cross-sectional studies [7,27], and with a study in a prospective birth cohort [26].

Current vaccination programs for children are complex and vaccinations are often multiple, both in terms of the content and by combination of more than one injection. Since vaccines also to some extent may vary regionally and among families who require individualized vaccination for their children, there is great difficulty in studying the effect of individual vaccines. We therefore chose to use several measures in order to investigate effects of vaccinations on the development of sensitization.

There was a significantly lower sensitization rate in the anthroposophic group, which is consistent with results in the whole ALADDIN cohort [9]. The comparison group from the non-anthroposophic families was representative for Swedish children both regarding sensitization and vaccination patterns, which allows for generalization of our findings [28]. In contrast to other similar studies [14, 15,20,26], the number of children with an individualized vaccination program was high and children unvaccinated up to age five years uniquely high.

This study has several strengths. The prospective design of this study, where exposure data were recorded before the outcome data became available, minimized the risk of recall bias or reverse causation, which in both cross-sectional and retrospective cohort studies complicates the assessment of causality [23]. Families were recruited during pregnancy and data collected longitudinally up to five years of child age. Presence of allergen-specific IgE is not always necessary for allergic disease, but often associated therewith [30]. It has the advantage of being an entirely objective measure, which is not the case for allergic disease, as this can be clinically defined or assessed in different ways.

There are some weaknesses in the study, which need to be considered. The information about lifestyle was based mainly on parental reports and thus subjective. A higher proportion of families in the anthroposophic group did not consent to blood sampling. However, selection bias is unlikely, as this reluctance to participate in blood sampling was not likely to be related to sensitization of the child or parent. The ALADDIN cohort constitutes a relatively limited study population – albeit well-characterized and with distinct differences in allergic sensitization between the lifestyle groups. Notably, the co-variation in exposures related to anthroposophic lifestyle, including delayed or avoided vaccination, results in limited statistical power to disentangle any relationship for the reduced sensitization in their children.

In summary, we found no support for an association between several aspects of vaccination (age at first vaccination; total sum of vaccine injections and of vaccines; MMR vaccination) and allergic sensitization. The reduced prevalence of such sensitization in the anthroposophic group does not seem to be explained by delayed or avoided vaccination in early childhood. Further studies concerning the components of the allergy-protecting capacity of the anthroposophic environment are called for. It would be valuable to assess allergic sensitization also in other groups with deviating vaccination patterns as well as to study clinical allergy diseases.

Contributors

JS, BA, FL, HJP, AS, GP, and JA were involved in the study design, the practical and theoretical work and carefully revised the manuscript. JS, BA, FL, and JA prepared the manuscript. JS and JA performed the statistical analyses with contributions from HJP. All authors have approved the final version for publication.

Declaration of Interests

Jackie Swartz is involved in the anthroposophic community.

Johan Alm has served as a consultant on clinical trials for ALK-Abello. There is no other relevant conflict of interests from any of the co-authors. A. Scheynius is a member in the Joint Steering Committee for the Human Translational Microbiome Program at SciLifeLab/Karolinska Institutet together with Ferring Pharmaceuticals, Switzerland.

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