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No epidemiological evidence for infant vaccinations to cause allergic disease

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Abstract

Context: The prevalence of allergic diseases has increased considerably over the last decades. The hygiene hypothesis has emerged, linking reduced microbial exposure and infections early in life with the development of allergic diseases. Especially some of currently available non-replicating infant vaccines are unlikely to mimic a natural infection-mediated immune response that protects against the development of allergic diseases. Moreover, several studies suggested infant vaccinations to increase the risk of allergic diseases. *Objective*: To determine whether infant vaccinations increase the risk of developing allergic disease. *Data Sources*: We searched MEDLINE from 1966 to March 2003 and bibliography lists from retrieved articles, and consulted experts in the field to identify all articles relating vaccination to allergy. *Study Selection and Data Extraction*: We selected epidemiological studies with original data on the correlation between vaccination with diphtheria, pertussis, tetanus (DPT), measles, mumps, rubella (MMR) and Bacillus Calmette-Guérin (BCG) vaccine in infancy and the development of allergic diseases, and assessed their quality and validity. *Data Synthesis*: Methodological design and quality varied considerably between the studies we reviewed. Many studies did not address possible confounders, such as the presence of lifestyle factors, leaving them prone to bias. The studies that offer the stronger evidence, including the only randomized controlled trial at issue published to date, indicate that the infant vaccinations we investigated do not increase the risk of developing allergic disease. Furthermore, BCG does not seem to reduce the risk of allergies. *Conclusions*: The reviewed epidemiological evidence indicates that, although possibly not contributing to optimal stimulation of the immune system in infancy, current infant vaccines do not cause allergic diseases.

Keywords: Childhood vaccination; Allergic disease; Hygiene hypothesis

1. Introduction

The prevalence of allergic diseases such as atopic asthma, hay fever and atopic eczema has increased considerably over the past decades [1–6]. The largest increase was reported from the United States, where self-reported prevalence rates for asthma increased by 75% from 1980 to 1994 [7]. While critical appraisals have shown the difficulty of defining objective measures of asthma and allergic disease in childhood, the increase nevertheless appears to be real [8–10]. Furthermore, allergic diseases are most common in Western, industrialized countries [11]. In 1989, Strachan proposed an explanation for the rising prevalence of allergic disease [12]. He postulated that microbial exposure and infections in early childhood prevent the development of allergic diseases, a proposition known as the hygiene hypothesis [12]. Since then, many epidemiological findings have supported this theory. For instance, living in a large family, attending day care early in life, and growing up on a farm or in a family with an anthroposophic lifestyle have shown to reduce the risk of asthma and allergies [13–17]. Recently, being exposed to food-borne and oro-fecal infections at early age was found to be associated with a decreased prevalence of hay fever and asthma [18]. These findings emphasize that living conditions as mentioned above probably predispose to infection and exposure to microbial structures, rather than being direct determinants of allergic disease [14]. In the

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previous century, improved sanitation and clean drinking water reduced the burden of many infectious diseases. However, a modern hygienic Western lifestyle may contribute to the development of atopic disease by depriving the developing immune system of essential microbial stimuli [18,19]. The identification of T-helper 1 and 2 lymphocyte subsets (Th1, Th2) with their typical cytokine patterns provided an immunological mechanism underlying the hygiene hypothesis. A predominance of Th2-type mediated responses is implicated in the development of allergic disease [20]. In newborns, the immune response is skewed away from a Th1 profile [21], reflecting the Th2 conditions of pregnancy [22]. Rapid suppression of Th2 responses and deviation towards a Th1 lineage can be seen in non-atopic infants [23]. Infants with genetic predisposition for the development of atopy can be programmed to consolidate Th2 responses to allergens within the first 6 months of life [21,23,24]. However, early exposure to certain viral respiratory infections, and to commensals and pathogens from gut flora appears to drive maturation of Th1-type responses [25–27].

Vaccination with current infant vaccines may contribute to limited induction of Th1 responses [28]. After completing a primary immunization series of acellular *Bordetella pertussis* vaccine (aP), mixed Th1–Th2 responses were found [29], whereas recipients of whole-cell vaccine (wP) showed only a Th1-type response [30]. Responses to tetanus toxin (TT) after a primary series of diphtheria, tetanus and acellular pertussis (DTaP) vaccination showed a steady Th2-type response that decreased after priming, but resurged after the age of 1 year [31]. After measles vaccination, infants showed reduced Th1 cell responses [32,33], which may reflect immunosuppression similar to that seen after natural measles infection in children [34]. Infants vaccinated at birth with Bacillus Calmette-Guérin (BCG) vaccine develop Th1-type immune responses to purified protein derivative (PPD) [35], whereas a Th2-type responses predominate in unvaccinated infants [36]. Early BCG vaccination, however, does not drive immune responses to other, unrelated vaccines towards a Th1-type response [37]. Vaccination against hepatitis B at birth, and at 2 and 4 months of age, with concomitant BCG vaccination at birth increased not only Th1 activity, but also a Th2-type response to HBsAg, compared to non-BCG vaccinated controls. BCG vaccination at birth followed by DTP vaccination at the ages of 2, 3, and 4 months also slightly but significantly, increased a Th2-type response to TT when compared to controls [37]. Therefore, it seems that the influence of BCG vaccination on the immune response is more complex than just a mere polarization towards Th1 responses. In general, T cell responses following vaccination of human infants are characterized by limited Th1 responses although, contrary to murine responses, preferential Th2 polarization is uncommon [28]. While the causes of the increasing prevalence of allergic diseases remain to be further uncovered [14], infant vaccinations were suggested to increase the risk of allergic disease [38,39]. Consequently, the question is whether healthy children vaccinated at early age with current vaccines have increased risk of development of allergic disease. The aim of this review is to answer this question based on the best available epidemiological evidence.

2. Methods

We searched MEDLINE from 1966 to March 2003 for articles with the search terms vaccine, allergy, atopy, asthma, eczema, and hay fever. We included (Latin) synonyms and different spelling preferences in the search. In addition, we used the *related articles* function in PubMed, and hand-searched bibliographies of the articles initially found to identify more relevant studies. Furthermore, personal communications with authors and other experts in the field lead to the retrieval of additional published and unpublished studies. The search was not restricted to a certain language or study design. All reports of original data on the influence of DTP or MMR or one of their components, or BCG on the development of allergic diseases were selected for the review. The quality and validity of the studies was appraised using methodological design, sample size, clinical characteristics of exposed groups, documentation of timing of vaccination, length of follow-up, outcome measures, and statistical methods as criteria.

3. Results

The following section describes the available epidemiological evidence on the relation between vaccination against DTP, measles, and BCG on the one hand, and allergy and asthma on the other. Despite the fact that the search was not restricted to English language, we found no relevant studies published in other languages. For reasons of brevity, details of studies that are discussed in the following are summarized in the Tables 1–3, categorized by pertussis/DTP, measles, and BCG vaccine, respectively.

3.1. Pertussis and DPT

In 1994, a non-peer reviewed letter caused considerable commotion when the authors linked pertussis vaccination to the development of asthma. They reported a cross-sectional survey that showed the relative risk of asthma, after vaccination with wP vaccine, to be over five times that of controls not vaccinated against pertussis [38]. A causal link was suggested, although by their nature, cross-sectional studies cannot demonstrate causality. Increased prevalence of allergic disease in immunized children was also found in a small cross-sectional survey in New Zealand with only 23 non-immunized subjects [39]. As McIntyre et al. [40] commented the number of subjects classified as non-immunized was so small that if only one of them had developed asthma, statistical significance would have been lost. Aptly they

Table 1
Pertussis/DTP vaccine

Ref. #, 1st author	Study design; time sequence, sampling method	Total number of subjects (*n*)	Immunizations, exposed group	Immunizations, control group	Mean age at vaccination (range)	Mean age at follow-up (range) (years)	Outcome measures								
							Atopic disease	Atopic disease Assessm.	Atopic dis. + vaccine+	Atopic dis. − vaccine+	Atopic dis. + vaccine−	Atopic dis. − vaccine−	*P*-value	Crude OR (95% CI)	Adjusted OR (95% CI)
[38], Odent	RET, CRS	446	At least wP	At least no pertussis	NA	7.87	Asthma	QST	26	217	4	199	<0.01	5.96 (2.04–17.4)	
[39], Kemp	RET, CHT	1.207[a]	Pertussis + DT, polio and measles	None[b]	3, 5 and 12–15 months	0–16	Asthma	QST, MRD	315	641	2	15	NS	3.69 (0.84–16.2)	
							Eczema	QST, MRD	398	552	7	12	NS	1.24 (0.48–3.17)	
							"Allergy"[c]	QST, MRD	404	530	1	12	0.010	9.15 (1.19–70.6)	[d]
[41], Farooqi	RET, CHT	1.855	At least wP	At least no pertussis	3, 5, and 9 months	12–16	Atopy	MRD	624	733	179	319	<0.01	1.52 (1.23–1.87)	1.76 (1.39–2.23)[e]
[42], Aaby	RET, CHT	400	Pertussis + DT, polio	NA	1–4 months	6.2 (3–14)	Allergic sensitation[f]	SPT	65	211	40	84	NS	0.65 (0.40–1.03)	2.88 (1.01–9.02)[g]
[43], Hurwitz	RET, CRS	13.612	DTP or tetanus[h]	At least no DTP or tetanus	NA	0.17–16	Asthma	QST	996	12329	10	274	0.016	2.2 (1.17–4.17)[i]	2.0 (0.95–6.74)[j]
							Hay fever	QST	470	12858	2	282	0.013	5.15 (1.28–20.8)	0.82 (0.16–4.35)
							Wheezing	QST	2776	10551	71	213	NS	0.79 (0.60–1.04)	1.23 (0.78–1.95)
							Any allergy ever	QST	2004	11282	16	268	<0.01	2.97 (1.79–4.94)	1.66 (0.67–4.14)
							Recent all symptoms	QST	5790	7519	112	172	NS	1.18 (0.93–1.50)	1.63 (1.05–2.54)
							Any allergy ever/recent all symptoms	QST	6347	6945	117	167	0.031	1.30 (1.03–1.66)	1.69 (1.1–2.59)
[44], Golding	PRO, CHT	12.692	At least wP	No pertussis	NA	5	Wheezing	QST	1500	10351	149	692	<0.01	0.67 (0.56–0.81)[k]	
							Eczema	QST	845	11006	96	745	<0.01	0.60 (0.48–0.74)	
							Hay fever	QST	295	11556	23	818	NS	0.91 (0.59–1.40)	
[45], Henderson	PRO, CHT	9.444	At least pertussis[l]	At least no pertussis	NA[m]	3.5	Wheezing	QST	770[n]	6897	101	810	NS	0.89 (0.72–1.11)	0.96 (0.72–1.16)[o]
[46], Nilsson	PRO, RCT	669	wP + DT	DT only	NA	2.4 (2.2–2.7)	Atopy[p]	QST, CLF, MRD, SPT	37	100	49	123	NS	0.93 (0.56–1.53)	0.91 (0.55–1.51)
			2-comp. aP + DT	DT only	NA	2.4 (2.2–2.7)	Atopy	QST, CLF, MRD, SPT	60	122	49	123	NS	1.23 (0.78–1.94)	1.20 (0.76–1.90)
			5-comp. aP + DT	DT Only	NA	2.4 (2.2–2.7)	Atopy	QST, CLF, MRD, SPT	55	123	49	123	NS	1.12 (0.71–1.78)	1.08 (0.68–1.72)
[47], Destefano	RET, CHT	167.240	wP + DT	At least no DTP	NA	2.3 (1.5–6)	Asthma	MRD, PDB	NA	NA	NA	NA	NA	NA	0.92 (0.83–1.02)
[48], Mullooly	RET, CCL	2.723	At least pertussis	At least no pertussis	NA	0–18 months	Wheezing	MRD, PDB	518	495	92	80	NS	1.18 (0.71–1.96)[q]	1.11 (0.61–2.00)
[49], Grüber	PRO, CHT	943	At least pertussis	At least no pertussis	NA	5	Asthma	QST, CLF	34	332	64	463	NS	0.74 (0.48–1.15)	
							Atopic dermatitis	QST, CLF	34	332	78	449	0.018	0.59 (3.8–0.90)	
							Hay fever	QST, CLF	18	348	29	498	NS	0.89 (0.49–1.62)	
							Allergic sensitation	RAST	79	159	134	234	NS	0.87 (0.62–1.22)	
[50], Anderson	RET, ECO	Approximately 745.000[r]	wP + DT	NA	NA	6–7 and 13–14	Wheezing	QST	NA	NA	NA	NA	0.047	−0.66 (−1.72–0.4)[s]	−0.53 (−1.49–0.43)[t]
							Hay fever	QST	NA	NA	NA	NA	0.002	−0.62 (−1.09–0.14)	−0.6 (−1.02–0.19)
							Eczema	QST	NA	NA	NA	NA	0.048	−0.37 (−0.9–0.16)	−0.27 (−0.76–0.21)

Totals may not add to *n*; see original papers for more details. Crude odds ratio and *P*-value were calculated based on numbers provided in or derived from text. List of abbreviations used: DT; diphtheria tetatnus vaccine, DTP; diphtheria tetanus pertussis vaccine PRO; prospective, RET; retrospective, CHT; cohort, CRS; cross-sectional, CCL; case-control study, RCT; randomised controlled trial, ECO; ecological study, aP; acellular pertussis vaccine, wP; whole-cell pertussis vaccine, QST; questionnaire, SPT; skin-prick test, MRD; medical record(s), PDB; pharmaceuticals database, CLF; clinical findings, NA; not available and NS; not statistically significant.

[a] The non-immunized group consisted of only 23 children, of whom 6 had missing data on asthma and allergic disease.

[b] No records of DPT before age 5 months and of immunization against measles at 12–15 months.

[c] Defined as consults for or episodes of rhinitis, food allergy or urticaria.

[d] Risk measures adjusted for known confounders unavailable as a result of small numbers of non-immunized children.

[e] Adjusted for the possible confounding affects of all the variables studied.

[f] Defined as a positive skin-prick test to any of the three allergens *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* or cockroach mix.

[g] Adjusted for BCG vaccination.

[h] Three hundred and thirty-two subjects from the total sample had missing data on vaccination status.

[i] Estimated from logistic regression model, weighted to account for the probability for selection, non-response, and age, sex and race composition of the USA.

[j] Adjusted for known confounders.

[k] Odds ratio of the development of wheezing after receiving at least one dose of pertussis vaccine.

[l] Text does not specify whether cellular or acellular pertussis vaccine was administered to study subjects.

[m] Mean age at vaccination not available, but 8358/9444 (85.5%) of the children in the study had been vaccinated against pertussis at the age of 6 months.

[n] The presented numbers and crude odds ratio are for the children with "recurrent wheezing" in the 31–42 months age group. Statistical analyses showed no significantly increased risk for any of the wheezing outcomes in vaccinated children.

[o] Relative risk of recurrent wheeze in all age groups after adjusting for potential confounding and modifying variables.

[p] Any of: asthma, eczema, hay fever, urticaria, or food allergy.

[q] Odds ratios of first onset of wheeze by "recency" of pertussis vaccination were estimated using a conditional logistic regression analysis model with documented onset dates, and showed no increased risks for any of the vaccination windows, relative to the 45+ days post-vaccination window. Data shown: No exposure versus exposure 45+ days prior to reference date.

[r] Ninety-one and 154 ISAAC centers with a median number of 2996 and 3064 children per center provided data on the 6–7 and 13- to 14-year-olds, respectively.

[s] Spearman rank correlation coefficients with 95% confidence intervals shown in the table represent the 13- to 14-year-old age group that show a significant negative association of DTP vaccine with wheezing, hay fever and eczema; other associations in the 13- to 14-year-age group or in the 6- 7-year-age group were not found.

[t] Adjusted for per capita gross national product (GNP).

Table 2
Measles vaccine

Reference first author	Study design; time sequence, sampling method	Total number of subjects	Immunizations, exposed group	Immunizations, control group	Mean age at vaccination (range)	Mean age at follow-up (range)	Outcome measures								
							Atopic disease	Atopic disease assessment	Atopic dis. + vaccin.+	Atopic dis. − vaccin.+	Atopic dis. + vaccin.−	Atopic dis. − vaccin.−	*P*-value	Crude OR (95% CI)	Adjusted OR (95% CI)
[17], Alm	RET, CRS	608[a]	At least MMR	At least no MMR[b]	18 months	8.6 (5–13) years	Atopy	SPT, IgE	118	236	63	191	<0.025	1.52 (1.06–2.17)	1.49 (1.01–2.17)[c]
[41], Farooqi	RET, CHT	1855	At least measles	At least no measles	13 months	12–16 years	Atopy	MRD	730	929	78	118	NS	1.19 (0.87–1.63)	
[42], Aaby	RET, CHT	400	At least measles vaccine	Natural infection	9 months	6.2 (3–14) years	Allergic sensitation[d]	SPT	74	224	31	71	NS	0.76 (0.46–1.24)	1.01 (0.54–1.93)
[44], Golding	PRO, CHT	12692	At least measles vaccine	At least no measles vaccine	12–15 months	5	Wheezing	QST	1439	6001	1016	4236	NS	1.00 (0.91–1.09)	
							Eczema	QST	868	6572	613	4639	NS	1.00 (0.89–1.12)	
							Hay fever	QST	327	7113	231	5021	NS	1.00 (0.84–1.19)	
[47], Destefano	RET, CHT	167240	At least MMR	At least no MMR	NA	12–15 months	Asthma	MRD, PDB	NA	NA	NA	NA	NA	NA	0.97 (0.96–1.23)
[48], Mullooly	RET, CCL	2.723	At least MMR	At least no MMR	NA	0–18 months	Wheezing	MRD, PDB	20	20	854	851	NS	1.02 (0.44–2.35)[e]	0.89 (0.30–2.62)
[49], Grüber	PRO, CHT	943	Measles and mumps	At least no measles or mumps	NA	5 years	Asthma	QST, CLF	84	718	14	71	NS	0.59 (0.32–1.10)	
							Atopic dermatitis	QST, CLF	92	710	19	66	<0.01	0.45 (0.26–0.78)	
							Hay fever	QST, CLF	35	767	10	75	<0.01	0.34 (0.16–0.72)	
							Allergic sensitation	RAST	189	365	21	27	NS	0.67 (0.37–1.21)	
[50], Anderson	RET, ECO	Approximately 745000[f]	At least measles	NA	NA	6–7 and 13–14 years	Hay fever	QST	NA	NA	NA	NA	0.015	−0.55 (−1.1–0.01)[g]	−0.47 (−0.98–0.04)[h]
							Eczema	QST	NA	NA	NA	NA	0.036	−0.50 (−1.1–0.1)	−0.42 (−0.98–0.13)
[51], Shaheen	RET, CHT	262	At least measles vaccine	No measles vaccine but natural infection	3 years	17.8 (14–21) years	Allergic sensitation	SPT	33	96	17[i]	96	0.043	1.94 (1.01–3.71)	2.78 (1.28–5.89)[j]

Totals may not add to *n*; see original papers for more details. Crude odds ratio and *P*-value calculated based on numbers provided in or derived from text. List of abbreviations used: MMR; measles mumps rubella vaccine, OR; adds ratio, CI; confidence interval, PRO; prospective, RET; retrospective, CHT; cohort, CRS; cross-sectional, CCL; case-control study, ECO; ecological study, SPT; skin-prick test, QST; questionnaire, IgE; specific IgE antibodies against common allergens, MRD; medical record(s), PDB; pharmaceuticals database, CLF; clinical findings, NA; not available and NS; not statistically significant.

[a] Number of children with information on atopy and vaccination status, total number of subjects in study was 675.

[b] Of the children at Steiner schools, only 52/295 (18%) had received MMR vaccine, but 180/295 (61%) of these mostly non-MMR vaccinated Steiner children had had natural measles infection.

[c] Adjusted for heredity and sex.

[d] Defined as a positive skin-prick test to any of the three allergens *D. pteronyssinus*, *D. farinae* or cockroach mix.

[e] Odds ratios of first onset of wheeze by "recency" of MMR vaccination were estimated with a conditional logistic regression analysis model using documented onset dates, and showed no increased risks for any of the vaccination windows, relative to the 45+ days post-vaccination window. Data shown: No exposure versus exposure 45+ days prior to reference date.

[f] Ninety-one and 154 ISAAC centers with a median number of 2996 and 3064 children per center provided data on the 6–7 and 13- to 14-year-olds, respectively.

[g] Spearman rank correlation coefficients with 95% confidence intervals shown in the table represent the 13- to 14-year-old age group that show a significant negative association of measles vaccine with hay fever, and eczema; other associations in the 13- to 14-year-age group or in the 6- to 7-year-age group were not found.

[h] Adjusted for per capita gross national product (GNP).

[i] Non-vaccinated children did experience natural measles infection.

[j] OR of development of atopy after measles vaccination as compared to measles infection, adjusted for "possible" confounders according to authors. However, a probable confounder has been that Th2-type immunity is associated with both increased prevalence of atopy and death by natural measles infection, leading to over-representation of infants with Th1-type immunity in the measles *infection* "survivors" group.

Table 3
BCG vaccine

Reference first author	Study design; time sequence, sampling method	Total number of subjects	Immunizations exposed group	Mean age at vaccination (range)	Mean age at follow-up (range)	Outcome measures								
						Atopic disease	Atopic disease assessment	Atopic dis. + vaccin.+	Atopic dis. − vaccin.+	Atopic dis. + vaccin.−	Atopic dis. − vaccin.−	*P*-value	Crude OR (95% CI)	Adjusted OR (95% CI)
[42], Aaby	RET, CHT	400	At least BCG	12 (0–3072) days[a]	6.2 (3–14) years	Allergic sensitazion	SPT	57	214	21	32	<0.01	0.41 (0.22–0.76)	0.19 (0.06–0.59)[b]
[44], Golding	PRO, CHT	12692[c]	At least BCG	NA[d]	5	Wheezing	QST	175	693	2384	9440	NS	1.0 (0.84–1.19)	
						Eczema	QST	102	766	1389	10435	NS	1.0 (0.81–1.24)	
						Hay Fever	QST	36	832	490	11334	NS	1.0 (0.71–1.41)	
[50], Anderson	RET, ECO	Approx. 745000[e]	Tuberculosis	NA	6–7 and 13–14 years	Wheezing	QST	NA	NA	NA	NA	NS	NA	[f]
						Hay fever	QST	NA	NA	NA	NA	NS	NA	
						Eczema	QST	NA	NA	NA	NA	NS	NA	
[54], Shirakawa	RET, CHT	867	At least BCG[g]	6 years	12 years	Symptoms of atopy	QST	136	154	29	46	NS	1.40 (0.83–2.35)	
						Atopy[h]	IgE	190	100	46	29	NS	1.20 (0.71–2.02)	
[55], Alm	RET, CHT	574	At least BCG	17 (0–180) days	5.5 (3.1–7.2) years	Symptoms of atopy[i]	QST, CLF	77[j]	139	145	213	NS	0.81 (0.57–1.15)	
						Allergic sensitazion	SPT	26	190	35	323	NS	1.26 (0.74–2.16)	
						Atopy	IgE	61	155	84	274	NS	1.28 (0.87–1.88)	
[56], Strannegard	RET, CRS	6497	At least BCG	Before age 1 year	8.0 (4–9) years	Allergy[k]	QST	49	183	1324	4756	NS	0.96 (0.70–1.33)	
						Allergy[l]	QST	8	54	34	89	0.026	0.39 (0.17–0.90)	
[57], Grüber	PRO, CHT	774	At least BCG	30 (1–343) days[m]	7 years	Atopic dermatitis	QST	27	65	241	441	NS	0.76 (0.47–1.22)	
						Recurrent wheezing	QST	31	61	211	471	NS	1.13 (0.71–1.80)	
						Allergic rhinitis	QST	30	62	165	517	NS	1.52 (0.95–2.42)	
[58], Grüber	RET, CHT	38808	At least BCG	NA	6 years	Atopic dermatitis	QST	2548	17835	1695	16730	<0.01	1.41 (1.32–1.50)	0.99 (0.87–1.13)
						Bronchial asthma	QST	1019	19364	940	17485	NS	0.98 (0.89–1.07)	0.85 (0.71–1.00)
						Hay fever	QST	367	20016	332	18093	NS	1.00 (0.86–1.16)	0.97 (0.73–1.29)

Totals may not add to *n*; see original papers for more details. Immunizations control group: at least no BCG. Crude odds ratio and *P*-value calculated based on numbers provided in or derived from text. List of abbreviations used: BCG; Bacille Calmette Guérin vaccine, PRO; prospective, RET; retrospective, CHT; cohort study, CRS; cross-sectional study, ECO; ecological study, QST; questionnaire, IgE; specific IgE antibodies against common allergens, SPT; skin-prick test, CLF; clinical findings, NA; not available and NS; not statistically significant.

[a] Median age of BCG vaccination 12 days, range 0 days to 8 years and 5 months.

[b] OR of development of atopy after (documented) BCG vaccination vs. after no BCG (not documented and no scar), adjusted for "possible" confounders.

[c] Of the total cohort, 868 children were vaccinated with BCG before the age of 5 years.

[d] Four hundred and seventy-three of 868 children (54.5%) had received BCG vaccination during the first week of life.

[e] Ninety-one and 154 ISAAC centers with a median number of 2996 and 3064 children per center provided data on the 6–7 and 13- to 14-year-olds, respectively.

[f] No significant associations of immunization against tuberculosis with wheezing, hay fever or eczema were found either in the in the 13–14 year or in the 6- to 7-year-age group.

[g] Exposed: the subgroup of tuberculin negative responders at age 6 with subsequent BCG vaccination, but still tuberculin negative at age 12. "Controls": positive responders at age 6 (therefore without subsequent BCG vaccination), but tuberculin negative at age 12 (see text for additional information).

[h] As defined by "high" IgE concentrations or positive antigen-specific IgE.

[i] At least one clinical symptom or history of bronchial asthma, atopic dermatitis, allergic rhinoconjunctivitis, food allergy or urticaria.

[j] Prevalence of positive history or clinical symptoms consistent with atopy.

[k] Born in Sweden.

[l] Not born in Sweden.

[m] Median age at BCG vaccination 30 days.

pointed out that data were missing for 6 of the 23 children in this non-immunized group. Similar results were found in a retrospective follow-up study using a birth cohort of one single British general practice [41]. Vaccination with wP was found to be a statistically significant predictor of atopy, although the investigators remained cautious to draw conclusions because of the difficulty of inferring causality from correlation in retrospective follow-up studies. In a study initiated to investigate the prevalence of atopy in children who had been vaccinated with BCG in infancy (which is discussed hereafter), researchers also investigated the relation between DTP and polio vaccines and atopy [42]. After adjusting for the confounding effects of BCG vaccination, there was an increase in the prevalence of atopy provided that the more sensitive of outcome measures was used. In an American cross-sectional survey using data from a large health survey, DTP or single tetanus vaccination was found to be associated with a twofold increase in the prevalence of asthma [43]. Despite the large sample size of this study the cross-sectional design, the exclusive use of parent-reported information and a substantial number of subjects with missing data made this study prone to confounding, information and selection bias, compromising the strength of evidence. In a prospective study of almost 13,000 children born in 1 week in 1970 in England, Scotland and Wales, children who were vaccinated with wP vaccine were significantly less likely to have a history of wheezing or eczema at the age of 5 years than those also immunized, but not against pertussis [44]. The authors argued that allergic diseases were the reason for withholding pertussis immunization. The relation between vaccination against pertussis and wheezing illnesses in young children was studied prospectively by Henderson et al. [45]. They examined a large British cohort of 9444 children but found no significant increase of early or later-onset wheezing at the age of 42 months in the children vaccinated against pertussis. In a Swedish randomized, controlled trial, 669 children were randomized into four groups to receive two or five-component aP or wP vaccine. The control group received only DT-vaccine [46]. Incidence rates of atopic disease were similar in the four groups after adjustment for family history of atopic disease at the age of 2.5 years. Therefore, the in vitro differences between aP and wP vaccines [29] do not seem to translate into differences in the development of atopy [46]. DeStefano et al. [47] studied associations between childhood vaccinations and the occurrence of asthma in childhood in a cohort of 167.000 children in the United States of America. For DTP and MMR vaccine, the study showed similar risks of asthma in vaccinated and non-vaccinated groups. The percentage of children not receiving DTP vaccination was small (3.6%), and the median age of 28 months at follow-up was short. The diagnosis of asthma, the primary endpoint, was made at the median age of 11 months, but the authors failed to discuss the difficulty of diagnosing asthma at this age [9]. Interestingly, this study showed an increased prevalence of asthma in association with Hib and hepatitis B vaccination. (Although these vaccinations have become part of vaccination programs in many countries, they are beyond the scope of this article.) Another study used a matched case-control design to investigate a possible association between time having passed since vaccination and wheezing in full-term infants [48]. The risk of wheezing during the first 18 months of life was not associated with time passed since wP vaccination, or OPV, HIB or HBV vaccination. A recent prospective study shows reduced atopic dermatitis in children vaccinated against pertussis, and a tendency towards a reduction of asthma, hay fever, and allergic sensitation [49]. Data from the ISAAC study were used in an ecological study to find an epidemiological link between infant immunizations and the prevalence of atopic disease in childhood on a population level [50]. Standardized questionnaires were distributed in over 90 countries and used to determine rates of atopic disease, whereas DTP, measles and tuberculosis vaccination rates were obtained from the WHO and from the participating centers. On a population level, there was a negative correlation association between DTP immunization and wheezing, hay fever, and eczema, as well as between measles immunization and hay fever and eczema. Both of these correlations weakened after adjustment for gross national product but remained statistically significant.

3.2. Measles and MMR

In 1979, a measles epidemic struck Guinea Bissau with a case-fatality rate of 25%. Survivors of a study cohort of 395 pre-schoolers, that had not been infected were vaccinated against measles [51]. In 1994, skin-prick tests for atopy of 262 then adolescents showed that the prevalence of atopy in the group that survived measles infection was approximately half that of the group that was not naturally infected but vaccinated against measles. The investigators suggested measles infection to reduce, and measles vaccination to increase the risk of atopy. Others soon commented on a possible confounding bias in the study. Atopic sensitation, associated with increased risk of development of atopy, is also associated with decreased cellular immunity. Thus, the children with atopic sensitation had been more likely to die in the measles epidemic, causing them to be under-represented in the natural measles infection “survivors,” non-vaccinated group [52,53]. A younger cohort from the same Guinea-Bissau population was also studied but did not show a higher prevalence of atopy in the group vaccinated against measles [42]. The investigators argue that many of the younger children in the vaccinated group had possibly experienced subclinical natural measles infection during the epidemic, and that this may have been a confounder. In a large prospective study of a birth cohort of British children mentioned before the prevalence of allergic diseases at the age of five was related to immunization status [44]. The 7440 children vaccinated against measles had a risk of atopy similar to the risk of those not vaccinated against measles. In a Swedish cross-sectional survey, 295

children from elementary anthroposophic schools were compared to 380 children from neighboring control schools [17]. Of the children in the Steiner schools, only 18% had received MMR vaccine, versus 93% in the control schools. Significantly fewer children of the anthroposophic schools had a history of atopic symptoms compared to the control children, and skin-prick and blood tests of the anthroposophic children were also significantly less frequently positive than those of the control children. Having received MMR vaccine seemed to increase the risk of atopy, but with a lower confidence interval limit approaching the value of one the difference was hardly significant. In a study of a birth cohort of a British general practice mentioned before [41], measles immunization was not found to be associated with an increased risk of atopic diseases. In addition, a large American cohort study mentioned before showed no increased risk of asthma after MMR vaccination [47]. A large American case-control study (mentioned before) showed no association of risk of wheeze during infancy with time having passed since MMR vaccination [48]. Conversely, measles and mumps vaccination significantly reduced prevalences of atopic dermatitis and hay fever, with a tendency towards reduction of asthma and allergic sensitation in a recent prospective study mentioned before [49]. Moreover, a significant negative association between measles vaccination and the prevalence of hay fever and eczema was found in the large ecological study using ISAAC records (also described above) [50]. This protective effect of measles vaccination was reduced after adjustment for GNP yet remained significant. In summary, the available data indicate that measles vaccination is not associated with an increased risk of atopic disease.

3.3. BCG vaccination

Shirakawa et al. [54] studied the development of delayed-type hypersensitivity reactions after injection of tuberculin at the ages of 6 and 12 years old in 867 Japanese children. If the reaction was negative at age six, the subject was vaccinated with BCG. At the age of 12, the investigators asked for atopic symptoms and measured serum IgE and Th1- and Th2 cytokine concentrations. This retrospective follow-up study attracted attention when it showed that in positive tuberculin responders at the age of 12, the rate of atopic symptoms was one-third of that in 12-year-old negative tuberculin responders. The investigators suggested exposure and response to *Mycobacterium tuberculosis* to reduce atopic disease. However, there is no reliable way to distinguish cause from effect in such a cross-sectional study. It may even be more likely that a prevailing Th2-type immune response and subsequently increased prevalence of atopy is cause rather than effect of a decreased tuberculin response. Perhaps the most interesting comparison in this study was not emphasized, commented Strachan saliently [14]. This is the comparison of the group of children that was vaccinated against BCG but did not build cellular immunity against it to the group that was not vaccinated against BCG but was later tuberculin negative at the age of 12. This comparison more or less reflects the influence of only BCG vaccination on atopy, and it shows similar rates of atopic symptoms and similar serum IgE and cytokine concentrations. The strength of this evidence remains limited, however, because there was no control group that was not exposed to *M. tuberculosis*. A retrospective cohort study of children in Guinea-Bissau did find a protective influence of early BCG vaccination on the development of atopy, with a tendency towards a larger reduction in atopy with younger age of BCG vaccination [42]. In a Swedish retrospective study, early BCG vaccination did not effect the development of atopic disease [55], nor was the prevalence of allergy reduced in children BCG vaccinated within the first year of life in a Swedish cross-sectional study [56]. Stronger evidence is provided by Gruber et al. [57] who studied a German birth cohort prospectively from birth to the age of 84 months. The cohort was regularly examined for the development of atopic disease with measurement of IgE concentrations. After a transient effect up to the age of 24 months, there were no statistically significant differences between the BCG vaccinated and non-BCG vaccinated infants either in atopic manifestations or in the total IgE concentrations. These findings were supported by another large retrospective study of this group that investigated associations between early BCG vaccination and ethnicity on atopic manifestations [58]. They found a strong protective effect of non-German ethnicity against atopic manifestations, but no significant protective effects of early BCG vaccination. In addition, the large ecological study mentioned before did not show a correlation between vaccination rates against tuberculosis and the prevalence of symptoms of atopic diseases [50]. In conclusion, there is no convincing evidence of any effect of BCG vaccination on the development of allergy at this point in time. Methodological flaws, different vaccine strains and dosages used and varying ages at vaccination have been suggested to be responsible for the conflicting results of the studies investigating the question at issue in a recent review [59]. However, the varying results that have been found may also originate in a limited true effect of BCG on the risk of atopy. To overcome at least methodological weaknesses and minimize bias, investigators in The Netherlands are currently performing a large multi-center randomized controlled trial [60]. The results of this study will follow.

4. Discussion

Vaccinations in infancy were suggested to increase the risk of allergic diseases. While stimulating humoral responses, current infant vaccines may induce limited cellular responses [28], especially non-replicating vaccines. Based on the best available epidemiological evidence, however, we conclude that there is no convincing evidence that these immunological mechanisms translate into a contribution of infant

vaccinations against diphtheria, pertussis, tetanus, measles, mumps and rubella to the development of atopic diseases. Therefore, our review of current evidence strengthens earlier conclusions that these infant vaccinations do not increase their risk of development of allergic diseases [47,61–64]. Furthermore, we are of the opinion that at this moment, there is insufficient evidence to accept or reject a causal relation between early BCG vaccination and the development of allergic disease.

When evaluating effects of health care interventions, randomized controlled trials (RCTs) provide the most reliable information [65]. So far, only one RCT addressing the question at issue was done [46]. This is mainly because it is considered unethical to withhold vaccination to study subjects. Randomization is the only means of allocation that also controls for unknown and unmeasured confounders, which is why Cochrane reviews concentrate primarily on RCTs. The other epidemiological studies reviewed are all observational studies, which are prone to bias. Therefore, a great deal of discernment is needed to assess their validity [65]. Several problems occurred assessing validity of the studies reviewed. Additional information regarding life style factors should be known because a conscious choice of parents not to have their child vaccinated may go with other lifestyle factors that affect the development of atopy, leading to confounding bias. Furthermore, many authors did not report the vaccination schedules that were followed, yet the vaccination programs that are offered worldwide vary considerably [63]. This is important because the first 6 months of life represent a critical time window for the initiation of immunological changes leading to the development of atopy [21,23].

Although the Th1–Th2-paradigm has been helpful in understanding general principles of allergic disease, it oversimplified the immunological mechanisms underlying the hygiene hypothesis [66,67]. Helminth infections induce a strong Th2-type response [68], yet parasitic infections are inversely associated with atopy and atopic disease [69,70]. Therefore, it is probably incorrect to regard Th2 skewing as the decisive influence leading to allergic disease [71,72]. Current evidence indicates that regulatory T (Tr) cells are crucial to control pathogenic immune responses by producing cytokines such as IL-10 and TGFβ [73]. These regulatory cytokines can inhibit both Th1 and Th2 responses in experimental models [74,75]. Infectious agents and commensal flora of the gut may stimulate the production of Tr cells [76] that seem pivotal to control not only atopic disorders, but also autoimmune diseases [73,76]. There is a trend towards an association between allergic and autoimmune diseases in individual patients [77,78]. These observations are in accordance with the concept of common mechanisms underlying infection-mediated protection against allergy and autoimmunity. A disturbed Th1/Th2 balance early in life could explain the increase in atopic disorders by lack of Th1 promoting infections. However, deficient induction of Tr cells in the absence of infection explains the increasing prevalence of both atopic and autoimmune diseases. Another mechanism contributing to microbial protection against allergic disorders relates to dendritic cells (DCs) and Toll-like receptors (TLRs). DCs are efficient antigen presenting cells (APCs) that can stimulate naïve T cells and drive them into distinct classes of effectors [79,80]. When stimulated, DCs can produce cytokines that may down-regulate allergic responses depending on host factors [81,82]. The innate immune system therefore seems to mediate the protective effect of microbial stimulation against the development of allergic disease [83,84]. The limited induction of Th1 responses after vaccination early in life appears to result from suboptimal interactions between APCs and T cells in infancy [85–88]. The induction of adult-like Th1 responses early in life requires optimal activation of neonatal DCs [28]. DC activation and maturation is typically triggered by stimulation of TLRs expressed on their surface [89]. TLRs are receptors for various microbial components such as lipopolysaccharide, heat shock proteins and the CpG motif of bacterial DNA [90–94]. Since modern subunit vaccines mostly lack these microbial antigens, they may not activate DCs efficiently. Likewise, microbial antigens such as heat shock proteins seem to have an intrinsic capacity to trigger Tr cells [95,96]. As a result, the absence of microbial antigens from vaccines may also impair regulation of the adaptive immune response. Recent advances in understanding how cell-mediated immunity is regulated have indicated substantial differences between responses after natural infection and vaccination that may contribute to the limited induction of Th1 responses after vaccination. Infants with a positive family history of atopy have a reduced Th1 response capacity [97]. Vaccination of these genetically predisposed infants is unlikely to stimulate upregulation of Th1-type responses.

In the light of the hygiene hypothesis, the effects of vaccinations remain complex: they prevent natural infection or modify its course, saving the lives of approximately 3 million children per year [98]. Although infant vaccination does not underlie the rise in allergic diseases, it may not stimulate the immune system optimally early in life. Therefore, the challenge is to construct vaccines that not only prevent infectious disease, but also mimic infection-mediated immune stimulation to protect against the development of allergic and autoimmune disease. In vaccines for preventing infectious diseases, non-replicating agents are preferred because of their safety in the normal and immunocompromised host. However, as non-replicating vaccines inherently lack immunogenicity, they commonly require adjuvants to induce an immune response [99]. Conventional vaccines have been designed primarily to induce antibodies, using classical adjuvants such as oil-based emulsions and aluminum salts to prolong release of antigen [61]. The increased humoral response induced by aluminum salts is associated with a predominant Th2-type immune response [100]. After a primary series of adsorbed–adsorbed diphtheria–tetanus (DT) vaccination, children boosted with adsorbed–adsorbed DT showed increased IgE responses to TT compared to non-adsorbed DT booster receivers at the age of 10 years [101]. However,

this did not translate into increased atopic symptoms in the aluminum-adsorbed DT group at the age of 12 [102]. Recently, efforts have concentrated on designing vaccine adjuvants that induce broad immune responses including humoral and cellular immunity, thus preventing preferential Th2 skewing of the immune response [103]. Vaccines that target DCs to provide protective antigens for presentation, and induce maturation and mobilization of DCs could contribute to such a balanced immune response [104]. In murine models, DNA sequences containing CpG motifs, heat shock proteins, as well as other adjuvants such as Freunds' complete adjuvant, and MF59 can successfully induce Th1 and regulatory cytokine responses early in life [28,105,106].

The prevention of infectious diseases by vaccination is one of the greatest achievements of biomedical science and public health [107]. Yet, worldwide, public health systems are facing increasingly complex immunization schedules in an era of waning public faith in the vaccine safety [108–110]. With a substantial portion of the population receiving vaccines in infancy, it is inevitable that many allergic diseases will appear after vaccination. As the causes of the increase of allergic diseases remain unclear, it is understandable that parents of vaccinees may suspect that sequence is consequence [111]. Ever since the times of Jenner the safety of vaccines has been mooted, but the controversy has soared in the last decade [112]. Parents searching the Internet for information are likely to encounter elaborate anti-vaccination sites. Many of these use rhetorical appeals and make explicit but unjust claims about vaccines [113,114]. The media can strongly sway public opinion about vaccination [115]. Therefore, to hold on to vaccines for the prevention of infectious diseases in the future, it is crucial to educate parents of vaccine recipients and those delivering vaccines now about what vaccines do and not do, and that a temporal sequence does not necessarily prove causality [111,116]. We conclude that current epidemiological evidence indicates infant vaccines do not increase the risk of allergic disease.

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