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Does BCG vaccination protect against the development of childhood asthma? A systematic review and meta-analysis of epidemiological studies

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Background Results have been conflicting as to whether Bacillus Calmette-Guérin (BCG) vaccine, a non-specific stimulator of the immune function, protects, predisposes or is unrelated to the development of childhood asthma. In this systematic review and meta-analysis, we qualitatively and quantitatively appraised the epidemiological evidence.

Methods Eligible studies were identified using a search strategy that included a computerized literature search and a manual search of each article's reference list, up to June 2008. A total of 23 studies were included (10 cohort, 5 case–control and 8 cross-sectional). Each study was summarized and rated for methodological quality. Pooled odds ratio (OR) estimates and 95% confidence intervals (CIs) were calculated using fixed-effects (FE) or random-effects (RE) models; if heterogeneity was present, the latter was used. Three indicators of BCG exposure were considered including BCG vaccination, tuberculin response and scar diameter.

Results The pooled estimate of association for 23 studies reporting on any of the three indicators suggested a protective effect of BCG exposure on childhood asthma occurrence. The studies were heterogeneous, especially when tuberculin response was considered. Restriction to a subgroup of 16 studies that considered BCG vaccination indicated a protective effect with no evidence of heterogeneity. The overall pooled OR using an FE model was 0.86 (95% CI 0.79–0.93). Exclusion of three studies with the lowest quality scores showed a similar association.

Conclusion These results strengthen the epidemiological evidence in support of the hypothesis that exposure to the BCG vaccine in early life prevents asthma, possibly through a modulation of the immune maturation process.

Keywords Asthma, BCG vaccine, child, meta-analysis, review, tuberculin

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Introduction

Asthma is the most common chronic disease in childhood and its increasing prevalence over the last 30 years[1] represents a major public health concern. Childhood asthma inflicts a substantial burden on the individual in terms of quality of life, absenteeism from school and interference with physical activities, and on the family causing parental absenteeism from work and limitations in social-life engagements.[2] It also impacts on the health care system in terms of resources utilization costs.[3,4] One promising research avenue into asthma prevention could involve a modulation of the immune response through exogenous agents such as with anti-inflammatory or immunosuppressive drugs. However, there is a special interest in identifying approaches that would be more innocuous such as prophylactic and therapeutic vaccines. In particular, the Bacillus Calmette-Guérin (BCG) vaccine, primarily used in tuberculosis prevention and often administered shortly after birth, is a strong inducer of a T-helper 1 (T_H1)-type immune response. The BCG vaccine has been explored as a protective factor against the development of asthma, proposed to occur via an inhibition of T-helper 2 (T_H2) immune responses characteristic of asthma.[5,6] Other inducers of T_H1 type immune responses include exposure to other types of mycobacterial infection, such as *Mycobacterium tuberculosis* and mycobacteria other than tuberculosis, which were studied in relation to atopy.[7] Pertussis vaccination has been also considered as another example of exposures that predominantly evoke T_H1 immune responses and could prevent asthma development.[8]

The BCG vaccine has been administered extensively over the years and across the world, and is generally considered to be very safe. Most reactions following intra-dermal administration of this vaccine are generally mild and do not require treatment. The usual response is the development of erythema, and either a papule or ulceration, followed by a scar at the immunization site. Two common complications following BCG vaccination of infants are regional suppurative lymphadenitis and osteitis. These complications reflect biological differences among BCG vaccine strains.[9] Therefore, through its strong immuno-modulatory effects, and in light of its recognized safety and simplicity,[10] it is of high interest to understand whether BCG vaccination could prevent asthma through immune deviation in infancy towards a T_H1-type response.

Previous qualitative reviews of the literature on the possible protective role of BCG vaccination in early life against the development of asthma suggested either conflicting results[11] or no association.[12] A similar conclusion of no association was reached in a meta-analysis limited to five studies.[8] In order to synthesize the overall available evidence, we systematically reviewed all epidemiological literature on the postulated association between BCG vaccination and asthma and presented a qualitative and quantitative summary of the results. One important aspect of our work was to explore methodological aspects that might have influenced the measures of association.

Methods

Search strategy

A systematic and comprehensive bibliographic search for all available evidence up to June 2008 was performed. Publications were retrieved from MEDLINE and PubMed from 1955 onwards, from EMBASE from 1974 onwards and from the Cochrane Central Register of Controlled Trials. In addition, Library and Archives Canada was checked for relevant dissertations. In order to capture studies on BCG vaccination, either one of the following medical subject heading terms and keywords was used: 'BCG', 'vaccine', 'tuberculin test', 'purified protein derivative', 'Mantoux test', 'skin testing', 'tuberculin response', 'tuberculin reactivity' or 'scar'. Subject heading terms used to identify studies on asthma included 'asthma' or 'wheezing'. We then combined our searches, including results for any of the BCG vaccination keywords and any of the asthma keywords. We further limited our search to studies conducted among children, the earliest one dating back to 1997 when Shirakawa and colleagues reported on the association between tuberculin responses and asthma for the first time.[13] The functions 'related articles' in PubMed and 'find similar' in EMBASE were used as well. The titles and abstracts of each publication were initially reviewed to eliminate irrelevant ones including review papers, letters, ecological or animal-based studies, studies pertaining to allergic diseases other than asthma and studies conducted among adult populations. Manual searching of reference lists of pertinent articles was done and review papers were scanned to check for additional eligible studies. No restrictions on the inclusion of publications by language were applied; studies published in non-English language were translated into English.

Study selection

We opted for liberal inclusion criteria with respect to study design, which enabled us to investigate the effects of methodological differences in original studies on the reported results. Cross-sectional surveys, case–control and cohort studies, as well as randomized controlled trials were considered eligible. The following inclusion criteria were applied to each study: (i) studies had to report on the association between exposure to BCG and the development of asthma and/or wheezing; and (ii) studies had to report on this association among children aged $\leqslant 19$ years. Studies in which the subjects were older were only included if childhood asthma data were reported[14] or obtained from the authors who

confirmed data calculation.[15] For one study that met the inclusion criteria, but in which the published data were insufficient for our purpose, the original investigators were contacted and asked for additional information.[16]

Indicators of exposure to BCG vaccination

The included studies used various indicators for BCG exposure: BCG vaccination, tuberculin response and scar diameter. BCG vaccination refers to the actual event of receiving the BCG vaccine. The tuberculin response, as measured by a standard tuberculin skin test (TST),[17] represents a delayed-type hypersensitivity reaction, usually resulting from but not limited to BCG vaccination. The scar diameter, as measured in millimeters, is the size of the scar usually present at the site of BCG vaccination. It is considered as a proxy measure of a previous BCG vaccination event (post-vaccination scar) or of a delayed-type hypersensitivity reaction. These three exposure indicators were considered to be relevant, although BCG vaccination as confirmed by medical records was regarded as the most valid.

Outcome measures

To be included, studies had to report on BCG exposure in association with childhood asthma per se or one of the asthma symptoms such as wheezing. Wheezing was considered in the current meta-analysis because it is the most specific symptom for predicting asthma and is often used as a diagnostic criterion for a clinical definition of asthma in epidemiological studies with adequate sensitivity and specificity.[18] Both current wheeze (defined as a positive answer to 'Have you had wheezing or whistling in the chest in the last 12 months?'), and ever wheeze (defined as a positive answer to 'Have you ever had wheezing or whistling in the chest at any time in the past?') were included. No other respiratory symptoms such as night cough, exercise-induced wheezing or cold-induced wheezing were considered.

Data extraction

Using a data extraction form, one researcher (M.Z.) extracted study specific data and a second researcher (M.C.R.) double checked this information. For each study, the following information was entered into a customized database: first author's name, study aim, publication year, country, inclusion and exclusion criteria, sample size, population characteristics, age and gender of participants, participation rate, indicators of exposure (definition and assessment), age of subjects at exposure, asthma and/or wheezing outcomes (definition and assessment), adjustment variables, odds ratios (ORs) and 95% confidence intervals (CIs).

Quality assessment

The methodological quality of each study was independently reviewed and critically appraised by two researchers (M.Z., M.C.R.). Disagreements and discrepancies between the reviewers were identified and discussed and a final score was determined for each paper by consensus.

Each individual study was assigned an overall rating score using a validated 'epidemiological appraisal instrument' that involves a set of 26 questions relating to subject selection, measurement quality, data analysis and generalization of results.[19] Each question was scored as follows: 'yes = 2', 'partial = 1', 'no or unable to determine = 0' and 'not applicable' responses were noted as such. The overall score was calculated as the average of the scores for the 26 questions, excluding the 'not applicable' responses, resulting in a possible range of 0–2. The numerical scores were then transformed into qualitative categories representing studies of highest, intermediary and lowest qualities. The cut-off points for these categories were chosen so as to distinguish between the top 25% of the distribution (highest: score $\geqslant$1.4), the next 50% (intermediary: score = 1.2–1.3) and the bottom 25% (lowest: score $\leqslant$1.1). Before consensus was attained, there was ~70% agreement (16 out of 23 studies) between the two independent reviewers on the categorical scores.

Statistical analysis

From each study, the OR that reflected the greatest degree of control for potential confounders was extracted. When no adjustments were made, the crude OR was selected. Extra-computation was occasionally performed to calculate ORs and 95% CIs when only absolute numbers and percentages[20–23] or relative risks[24] were provided or when the BCG-exposed category was considered the reference for calculating the OR.[25] When the risk estimate for the outcome was reported for the same children at different ages,[13,26,27] the one reported at the oldest age within childhood was chosen. This was based on the literature relating to the natural history of asthma, which argues for more reliable and valid asthma diagnosis at older compared with younger ages during childhood, such as that <5–6 years old.[28,29] When ORs were reported for more than one exposure indicator or outcome measure, a hierarchical assignment criterion was applied. BCG vaccination was regarded as the most valid exposure measure, followed by tuberculin response and scar diameter. Asthma was regarded as the most valid outcome measure, followed by current wheeze and ever wheeze.

In the pooled estimates, each study was attributed a weight corresponding to the inverse of its variance. The ORs and corresponding standard errors (SEs) were transformed to their natural logarithms to stabilize the variances and to normalize the distributions. The SEs were derived from the CIs reported in each study. CIs presented in the figures were estimated based on the SEs and thus might slightly differ from those reported in original studies.

An overall pooled summary OR for the association between BCG exposure indicators (BCG vaccination, tuberculin response or scar diameter) and asthma was estimated using a random-effects (RE) model in situations where heterogeneity was present to incorporate the between-study component of variance in the weight.[30,31] Fixed-effect (FE) pooled estimates were preferentially used when heterogeneity was absent.[32] The FEs model assumes that there is no between-study variance, i.e. that the results of the studies are homogenous and that their variations are largely due to sampling. However, we present the results of both FE and RE models for the sake of comparison.

Statistical heterogeneity among studies was evaluated using the Cochran chi-square heterogeneity test Q, and the index of consistency I^2 statistics.[33,34] A two-sided $P < 0.1$ for the Cochran chi-square test was considered to be representative of heterogeneity.[35] I^2, used to quantify the extent of heterogeneity, is an estimate of the proportion of total variation in study estimates due to heterogeneity calculated as $100\% \times (Q - \mathrm{df})/Q$, where df is the degrees of freedom. Negative values of I^2 were put equal to zero, and values of 0, 25, 50 and 75% suggested no, low, moderate and high degrees of heterogeneity, respectively.[33] In addition, the Galbraith plot, where the z statistic (ratio of $\log_{OR}$ to its SE) is plotted against the reciprocal of the SE, was used to visually assess heterogeneity.[36] The pooled effect estimate is represented by the slope of the line through the origin and the 95% limits are positioned two units above and below this line. Points outside of the two 95% limit lines indicate the studies that contribute most to heterogeneity. A sensitivity analysis was performed by omitting each study at each time from the pool and recalculating the summary OR to assess whether any of the studies was overly influential in the meta-analysis.[37] Potential publication bias, or the possibility that unpublished data would contradict the results of the published studies, was checked by visually inspecting funnel plots and by using Egger's test.[38] Bias can be suspected if the plots are widely skewed versus a plot resembling an inverted triangle, which represents a lower likelihood of publication bias.

In addition, to investigate the consistency and the robustness of the findings, we stratified the analysis according to predefined methodological aspects that might have influenced the observed associations. These included study design, study quality, exposure indicators (BCG vaccination, tuberculin response or scar diameter), exposure ascertainment (relying on records, scar presence or reporting), outcome measures (asthma, 'current wheeze' or 'ever wheeze'), asthma ascertainment (relying on clinical assessment or reporting of medication use, or reporting of diagnosis/symptoms/ever having asthma) and adjusting for confounders (no, partial or comprehensive adjustments). Statistical analyses were carried out with Stata 9.0 (Stata Corp Lp, College Station, TX, USA).[39]

Results

Study search results

Figure 1 summarizes the identification and the selection process for studies included in the meta-analysis.

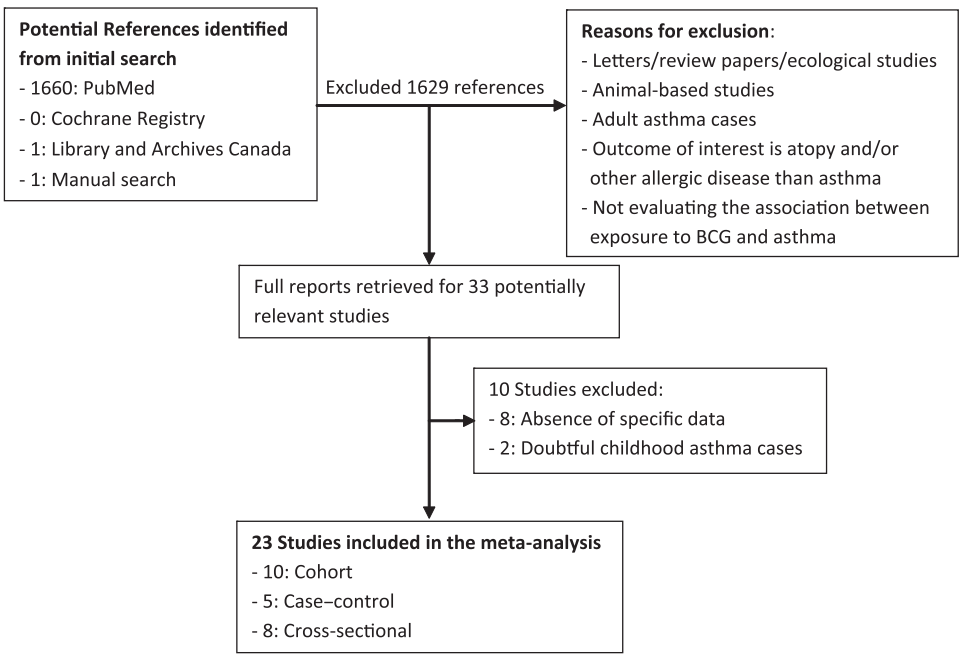

Figure 1 Flow chart of the identification and selection of eligible epidemiological studies examining the association between exposure to BCG and childhood asthma. The number of potentially relevant studies to be included in the meta-analysis is outlined as well as the reasons for exclusion

Table 1 Studies excluded from the systematic review and meta-analysis of the association between BCG exposure and childhood asthma

Author (reference), country	Design	Reason for exclusion
Steenhuis *et al.*,[43] The Netherlands	RCT	Very young subjects ($\leqslant$18 months), which prohibits reliable asthma diagnosis
Townley *et al.*,[44] Argentina, Thailand, Turkey	PC	No data reported specific to tuberculin response in association with asthma or wheezing Original investigators indicated very small numbers for wheezing and asthma
Bager *et al.*,[45] Denmark	RC	No indication on whether childhood or adulthood asthma cases Original investigators indicated that subjects were not asked about age of asthma onset
Martignon *et al.*,[46] France	RC	Data reported in combination with exposure to other vaccines (DTPolio, Pertussis) No data reported specific to BCG vaccination in association with asthma Original investigators indicated difficulty in providing requested data
Yilmaz *et al.*,[47] Turkey	CC	No data reported specific to tuberculin response in association with asthma Inability to reach original investigators
Ozmen *et al.*,[48] Turkey	CC	No data reported specific to BCG vaccination or tuberculin response in association with asthma Original investigators indicated difficulty in providing requested data
Strannegard *et al.*,[49] Sweden	CS	No data reported specific to BCG vaccination or tuberculin response in association with asthma Original investigators indicated difficulty in providing requested data
Castanon *et al.*,[40] Mexico	CS	No data reported specific to tuberculin response in association with asthma Inability to reach original investigators
Ma *et al.*,[42] China	CS	No data reported specific to the size of the scar diameter in association with asthma or wheezing Original investigators indicated difficulty in providing requested data
Ahmadiafshar *et al.*,[50] Iran	CS	No data reported specific to the size of the scar diameter in association with asthma Inability to reach original investigators

CC: case–control; CS: cross-sectional; PC: prospective cohort; RC: retrospective cohort; RCT: randomized control trial.

The initial search strategy yielded more than 1000 citations, which were mainly retrieved from PubMed; no additional studies were identified from EMBASE. After screening the titles and abstracts, we retrieved 33 potentially relevant studies for further review. Three studies were published in either Spanish,[40] Japanese[41] or Chinese,[42] and were translated into English. Out of the 33 studies, 10 were excluded mainly due to the absence of data specific to exposure–outcome associations of interest and the inability to obtain these data from the original investigators. Table 1 lists these studies along with selected characteristics and the reasons for exclusion (a detailed study description is provided in Appendix 1).

Study characteristics

Table 2 provides a detailed description of included studies, organized by study design and ordered by year of publication. The studies were published between 1997 and 2008 with most published after 2000. None of these studies was conducted on the same study population, nor consisted of re-analyses. Of these 23 studies, 10 were cohort studies, 5 were case–control and 8 were cross-sectional in design. The studies were conducted in several countries: three were conducted in each of Japan, Germany and Brazil, two in each of England and Australia and one study was conducted in each of Crete, South Africa, Turkey, Canada, Germany and The Netherlands, China, Estonia, Spain, New Zealand

Table 2 Summary of 23 epidemiologic studies included in the systematic review and meta-analysis of the association between BCG exposure and childhood asthma

Author (ref.), country	Age (years)	Exposure indicators	Exposure assessment	Age at exposure (years)	No. exposed (diameter size, mm)	No. unexposed (diameter size, mm)	Outcome measure	OR (95% CI)[a]	Outcome assessment	Adjustment variables	Quality Scores[b]
Prospective cohort											
Gruber *et al.*,[26] Germany	0.25–7	BCG vaccination	Official records	Within first year	92	682	Ever wheeze	**1.13 (0.71–1.79)**	Parental reporting of two or more episodes with shortness of breath		1.3
		Tuberculin response	TST	7	58 (⩾5)	432 (<5)	Ever wheeze	0.54 (0.13–2.32)			
Retrospective cohort											
Shirakawa *et al.*,[13] Japan	12–13	Tuberculin response	School records	6 and 12 (vaccinated after birth, 6 and 12 years)	288 (⩾10)	579 (<10)	Asthma	**0.42 (0.24–0.56)**	School-doctor diagnosis	Sex, lifestyle, nutritional status, environmental factors, family history, pet exposure, character and ventilation of homes, rural area residence	1.3
Alm *et al.*,[20] Sweden	2–7.2	BCG vaccination	Official records	Girls: mean = 17 days (range= 0–180) Boys: mean = 21 days (range = 1–173)	216	358	Asthma	**0.83 (0.48–1.43)**	Parental reporting plus doctor assessment of up to three wheezing episodes < 2 years or one episode at ⩾2 years, or any episode independent of age if with familial atopy or other atopic symptoms	Age matched cases and controls	1.2
Gruber *et al.*,[52] Germany	Mean = 6	BCG vaccination	Official records	At birth	20 383	18 425	Asthma	**0.85 (0.71–1.00)**	Parental reporting of present or past definitive diagnosis and/or characteristic symptoms	Sex, age, number of children at home, day care attendance, atopic dermatitis, hay fever	1.8
Marks *et al.*,[24] Australia	7–14	BCG vaccination	Personal health records + scar presence	(Policy is to vaccinate within 2 months of birth)	309	442	Asthma	**1.11 (0.82–1.50)**	Parental reporting of diagnosis or ever having medicines plus spirometry and methacholine challenge tests	Any positive skin prick test response, family history of asthma	1.3
							Current wheeze	1.06 (0.75–1.50)	Parental reporting		
							Ever wheeze	0.97 (0.72–1.32)	Parental reporting		
		Tuberculin response	TST	7–14	44 (⩾5)	263 (<5)	Asthma	0.62 (0.31–1.98)	Parental reporting of diagnosis or ever having medicines plus spirometry and methacholine challenge tests		

							Current wheeze	0.55 (0.23–1.29)	Parental reporting		
							Ever wheeze	0.54 (0.26–1.12)	Parental reporting		
Annus *et al.*,[27] Estonia	10–11	Tuberculin response	School records	2–3, 6–7, 10–11	153 (⩾5)	190 (<5)	Asthma	**0.24 (0.03–2.17)**	Parental reporting of diagnosis		1.5
							Current wheeze	0.36 (0.10–1.07)	Parental reporting		
							Ever wheeze	0.67 (0.38–1.17)	Parental reporting		
Benke *et al.*,[15] Australia	21–47 (asthma onset ⩽19)	BCG vaccination	Subject reporting	0–19	679	353	Asthma	**0.71 (0.52–0.96)**	Subject reporting of ever asthma		1.0
Garcia-Marcos *et al.*,[53] Spain	6–7	BCG vaccination	Area-based vaccination policy	At birth	6762	2828	Current wheeze	**0.87 (0.76–1.00)**	Parental reporting	Sex, age, younger/older siblings, current parental smoking, cat/dog exposure in first year, mother with university studies, truck traffic	1.3
Linehan *et al.*,[54] England	6–11	BCG vaccination	Official records	<0.25	1900	2535	Current wheeze	**0.68 (0.53–0.87)**	Parental reporting	Male sex, history of hay fever or eczema, family history of asthma, single adult households, adult smokers in home	1.3
Miyake *et al.*,[55] Japan	8–11	BCG vaccination	School records	Infancy	5567	150	Asthma	**0.68 (0.41–1.17)**	Parental and subject reporting of current wheeze plus ever asthma	Sex, grade, sibship size, smoking in household, paternal and maternal history of asthma, atopic eczema, allergic rhinitis, parental educational level	1.5
							Current wheeze	0.75 (0.48–1.23)	Parental and subject reporting		
		Tuberculin response	School records	6–7 years	2710 (⩾10)	2857 (<10)	Asthma	0.78 (0.64-0.95)	Parental and subject reporting of current wheeze plus ever asthma		
							Current wheeze	0.80 (0.67–0.94)	Parental and subject reporting		
Case–control											
Sarinho *et al.*,[56] Brazil	2–9	Scar diameter	Measurement of scar size	2–9	179 (⩾5)	89 (<5)	Asthma	**0.42 (0.24–0.74)**	Parental reporting of up to 3 dyspnea episodes in last 2 years plus responded well to oral or inhaled broncho-dilator		0.6

(continued)

Table 2 Continued

Author (ref.), country	Age (years)	Exposure indicators	Exposure assessment	Age at exposure (years)	No. exposed (diameter size, mm)	No. unexposed (diameter size, mm)	Outcome measure	OR (95% CI)[a]	Outcome assessment	Adjustment variables	Quality scores[b]
Wickens *et al.*,[57] New Zealand	7–9	BCG vaccination	General practitioner notes plus health records	(Available records since birth)	13	438	Asthma	**1.23 (0.41–3.72)**	Parental reporting of diagnosis plus current medication use		1.3
Samuel[14] Canada	18–48 First asthma attack <18 years	BCG vaccination	Official records	<1 to >1 (up until 11th grade)	61	107	Asthma	**1.10 (0.50–2.60)**	Subject reporting of history of characteristic symptoms plus clinical assessment by pulmonary function tests	Older age, male gender, serious respiratory infection in childhood, sibling with asthma	1.3
Mommers *et al.*,[16] Germany and Netherlands	7–8	BCG vaccination	Official records		67	257	Asthma	**1.16 (0.71–1.89)**	Parental reporting of asthmatic symptoms or coughing in the morning or during the day or evening in autumn and winter plus coughing daily for 3 months a year		1.3
							Current wheeze	1.02 (0.52–2.01)	Parental reporting		
							Ever wheeze	1.30 (0.78–2.18)	Parental reporting		
Queiroz *et al.*,[25] Brazil	6–14	Scar diameter	Measurement of scar size	6–14 (vaccinated at first month of birth)	141 (⩾5)	39 (<5)	Asthma	**0.25 (0.09–0.68)**	Parental reporting of three episodes of breathlessness that responded to broncho-dilators	Family history of atopic illnesses, skin test for mites	0.7
Cross-sectional											
Yoneyama *et al.*,[41] Japan	5–12	Tuberculin response	TST	5–6, 11–12	29 (diameter size not specified)	32	Asthma	**0.35 (0.03-3.52)**	Parental reporting (no specific diagnostic information reported)		1.1
Wong *et al.*,[58] China	10	Tuberculin response	Official records	Mean = 8.5 years (SD = 1.4) (vaccinated at birth)	359 (⩾10)	1842 (<10)	Asthma	**1.16 (0.78-1.72)**	Parental reporting of diagnosis	Sex, age	1.4
							Current wheeze	1.24 (0.80-1.93)	Parental reporting of diagnosis		
							Ever wheeze	0.99 (0.70-1.40)	Parental reporting of diagnosis		
Pahari *et al.*,[21] England	11–18	BCG vaccination	Reported + scar presence		195	113	Asthma	**0.81 (0.41–1.61)**	Parental reporting of diagnosis		0.8
		Tuberculin response	TST	11–18	217 (⩾10)	112 (<10)	Asthma	0.89 (0.46–1.72)	Parental reporting of diagnosis		

Da Cunha *et al.*,[59] Brazil	12–16	BCG vaccination	Presence of scar	(vaccinated at 0–4 years plus 50% revaccinated at school)	1089	523	Asthma	**0.83 (0.58–1.20)**	Subject reporting of ever asthma	Parental asthma	1.3
							Current wheeze	0.92 (0.64–1.32)	Subject reporting		
							Ever wheeze	0.99 (0.79–1.27)	Subject reporting		
Bibakis *et al.*,[22] Crete	7–18	BCG vaccination	Presence of scar	7–18 (vaccination policy at 6 years)	732	46	Ever wheeze	**2.33 (0.31–17.37)**	Parental reporting of atopic ever wheeze		1.3
		Tuberculin response	TST	7–18	644 (⩾3)	134 (<3)	Ever wheeze	1.35 (0.52–3.53)	Parental reporting of atopic ever wheeze		
Obihara *et al.*,[60] South Africa	6–14	Tuberculin response	TST	Mean = 10.3, inter-quartile range = 8.2–12.5 (vaccinated during first week of life)	327 (⩾10)	514 (<10)	Asthma	**0.72 (0.35–1.45)**	Parental reporting of ever and current symptoms	Sex, age, parental allergic history, average household income, no. of siblings, household environmental tobacco smoke, clustering	1.8
Mohrenschlager *et al.*,[61] Germany	5–7	BCG vaccination	Parental reporting plus official vaccination certificate		1243	253	Asthma	**0.70 (0.33–1.50)**	Parental reporting of diagnosis	Sex, age, parental education, passive smoking, nationality, playing predominantly outdoors	1.6
							Current wheeze	0.53 (0.25–1.12)	Parental reporting		
Soysal *et al.*,[62] Turkey	8 ± 4	BCG vaccination	Presence of scar		290	71	Asthma	**1.26 (0.56–2.83)**	Parental reporting of diagnosis		0.9
							Ever wheeze	1.22 (0.66–2.26)	Parental reporting		
		Tuberculin response	TST	Mean = 8 years (SD = 4)	236 (⩾10)	125 (<10)	Asthma	0.98 (0.52–1.86)	Parental reporting of diagnosis		
							Ever wheeze	0.77 (0.47–1.26)	Parental reporting		

SD: standard deviation.
[a]ORs and 95% CIs in bold refer to individual study estimates considered as the most valid when ORs and 95% CIs were reported for more than one exposure indicator and/or outcome measure.
[b]The highest quality studies had a score ⩾1.4, the intermediary had a score of 1.2–1.3 and the lowest had a score ⩽1.1.

and Sweden. The minimum age of subjects ranged from 0.25 to 12 years and the maximum age ranged from 7 to 18 years. Although two studies considered adult subjects, data were either reported[51] or obtained[15] on childhood onset of asthma.

As previously mentioned, three exposure indicators and three outcome measures were considered. With respect to exposure, 10 studies looked at the effect of BCG vaccination,[15,16,20,51–54,57,59,61] 5 considered tuberculin response,[13,27,41,58,60] 6 considered both BCG vaccination and tuberculin response,[21–24,26,55], whereas only 2 studies considered scar diameter.[25,56] BCG vaccination was mainly ascertained from records,[16,20,24,26,51,52,54,55,57,61] reported by subjects[15,21] or determined by the presence of a scar.[21–23,59] Tuberculin response was measured using TST,[21–24,26,41,60] or obtained from previously recorded TST results.[13,27,55,58] The scar diameter was ascertained using a simple millimeter ruler.[25,56] With respect to outcome, risk estimates were provided individually for both asthma and wheezing in 8 studies,[16,23,24,27,55,58,59,61] for asthma alone in 11 studies[13,15,20,21,25,41,51,52,56,57,60] and for wheezing alone in 4 other studies.[22,26,53,54] The asthma definition was based on clinical assessment,[13,20,24,51] medication use,[25,56,57] reporting of asthma previously diagnosed by a physician[21,23,27,52,58,61] or reporting symptoms or ever having asthma in the past.[15,16,41,55,59,60] As for wheezing, the majority of studies relied on parental reporting of wheezing symptoms. Regarding the quality of the studies included in the meta-analysis, 6 articles had the highest, 11 had intermediary and 6 had the lowest quality scores.

Table 3 Meta-analyses by indicators of BCG exposure

Exposure indicators	No. of studies	Exposed		Unexposed		FE summary OR (95% CI)	Measures of heterogeneity			RE summary OR (95% CI)
		n	%	*n*	%		*Q* (df)	*P* value	I^2 (95% CI)	
BCG vaccination	16	39 598	96.41	27 581	89.36	0.86 (0.79–0.93)	14.16 (15)	0.51	0 (0–52)	Same as FE
Tuberculin response	5	1156	2.81	3157	10.23	0.78 (0.59–1.05)	10.09 (4)	0.04	60 (0–85)	0.65 (0.36–1.17)
Scar diameter	2	320	0.78	128	0.41	0.37 (0.23–0.61)	0.76 (1)	0.38	NA[a]	Same as FE
Total	23	41 074	57.09	34 843	42.91	0.83 (0.77–0.90)	36.17 (22)	0.03	39 (0–63)	0.82 (0.72–0.92)

NA: not applicable.
[a]Df must be $\geqslant 2$.

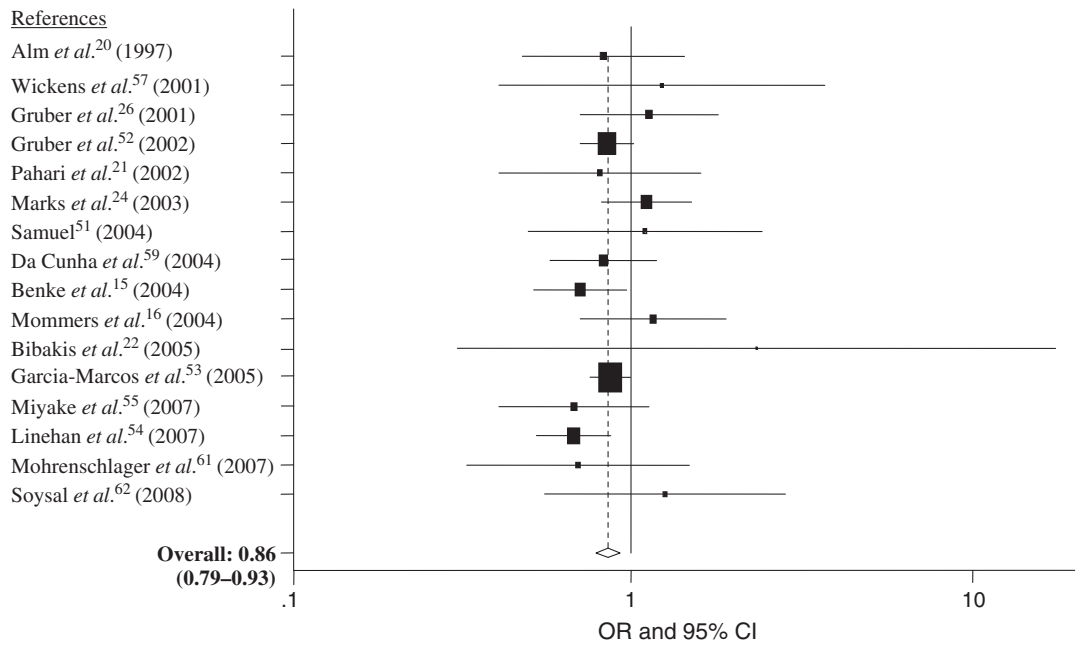

Figure 2 ORs and 95% CIs from 16 epidemiological studies examining the association between BCG vaccination and asthma. Studies are ordered by year of publication, the centre of each square represents study-specific ORs and the sizes of the squares reflect the statistical weight that each study contributed to the summary statistic (inverse of the variance); horizontal lines represent 95% CIs; vertical vertex of the diamond represents the OR summary estimate whereas the ends of the diamond (width) correspond to the 95% CI; dashed line is plotted vertically through the combined OR; vertical line indicates an OR of 1 (no difference)

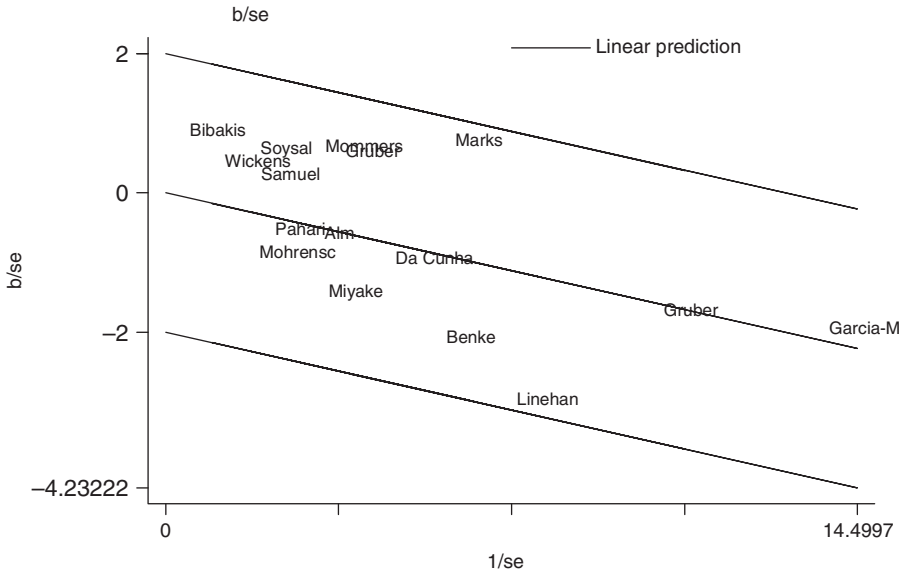

Figure 3 Galbraith plot for visual testing of heterogeneity among epidemiological studies examining the association between BCG vaccination and childhood asthma. The z statistic, which is the ratio of $\log_{OR}$ to its standard error (b/se), is plotted against the reciprocal of the standard error (1/se). The pooled effect estimate is represented by the slope of the line through the origin and the 95% limits are positioned two units above and below this line

Meta-analyses

Table 3 shows that the studies described a total of 75 917 participants divided into approximately similar proportions of subjects exposed and unexposed to BCG. The overall number of exposed participants across studies was 41 074 (57.09%); for ~96% the exposure of interest was BCG vaccination. A meta-analysis of all 23 studies reporting on any of the three indicators of exposure to BCG revealed that these studies were statistically heterogeneous ($Q = 36.17$, $P = 0.03$, $I^2 = 39\%$), suggesting that they cannot be pooled together. Heterogeneity was mostly apparent when tuberculin response was considered the indicator of exposure to BCG. Specifically, heterogeneity could be attributed to three studies[13,25,56] that were outside the 95% limits (Galbraith plot not shown). One of these studies considered tuberculin response and was seminal to the discussion of a potential protective effect of T_H1-inducing vaccines against allergy[13] and had an intermediary quality score. The other two, classified among the studies with the lowest quality scores, used the BCG scar size as an indicator of the BCG vaccination effect and observed a very strong protective association between the average diameter of the BCG scar and asthma.[25,56]

Because neither the tuberculin response nor the diameter size of the BCG scar is biologically equivalent to actual BCG vaccination, we further restricted analyses to the 16 studies in which exposure was BCG vaccination per se. As shown in Table 3, the 16 studies were found to be homogenous ($Q = 14.16$, $P = 0.51$, $I^2 = 0\%$). Using an FE model, the overall pooled OR was 0.86 (0.79–0.93), indicating a protective effect of BCG vaccination with the occurrence of asthma in childhood. Figure 2 shows a forest plot summarizing the study specific and pooled ORs and the 95% CIs of childhood asthma risk in relation to BCG vaccination. When examining the influence of each individual study on the overall model-specific summary effect estimates, the influence analysis indicated that no one single study affected the pooled OR when removed from the analysis (data not shown).

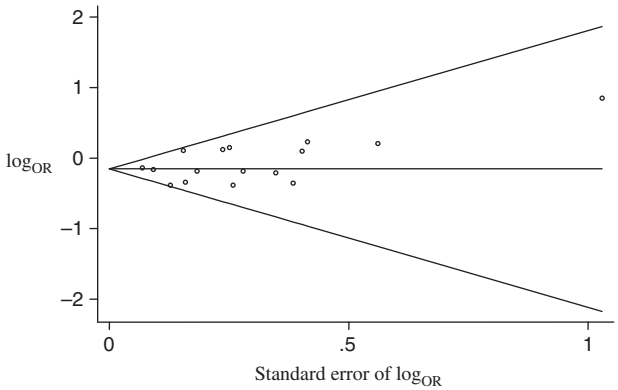

Figure 4 Funnel plot for publication bias among epidemiological studies examining the association between BCG vaccination and childhood asthma. The horizontal line indicates the FE summary estimate, whereas the sloping lines indicate the expected 95% CIs for a given SE, assuming no heterogeneity between studies. The small studies appear near the origin of the plot while the larger ones appear further right. The plot is nearly symmetrical, suggesting absence of severe publication bias

Table 4 Summary ORs of childhood asthma in association with BCG vaccination by methodological aspects

Methodological aspects	No. of studies	FE summary OR (95% CI)	Measures of heterogeneity			RE summary OR (95% CI)
			Q (df)	P value	I^2 (95% CI)	
Design						
Cohort	8	0.84 (0.78–0.92)	8.91 (6)	0.18	33 (0–71)	0.84 (0.75–0.94)
Case–control	3	1.15 (0.78–1.70)	0.03 (2)	0.99	0 (0–90)	Same as FE
Cross-sectional	5	0.86 (0.66–1.13)	2.81 (5)	0.73	0 (0–75)	Same as FE
Exposure ascertainment						
BCG—records	10	0.87 (0.77–0.97)	10.81 (9)	0.29	17 (0–58)	0.88 (0.76–1.00)
BCG—scar presence	4	0.89 (0.67–1.19)	1.80 (3)	0.62	0 (0–85)	Same as FE
BCG—reporting or area-based	2	0.84 (0.74–0.95)	1.38 (1)	0.24	NA[a]	0.83 (0.70–0.98)
Outcome measures[b]						
Asthma	12	0.87 (0.78–0.98)	8.40 (11)	0.68	0 (0–58)	Same as FE
Current wheezing	2	0.82 (0.73–0.93)	2.90 (1)	0.09	NA[a]	0.79 (0.62–1.00)
Ever wheezing	2	1.17 (0.74–1.84)	0.47 (1)	0.49	NA[a]	Same as FE
Asthma ascertainment[c]						
Clinical diagnosis or medication use	4	1.05 (0.82–1.34)	0.93 (3)	0.82	0 (0–85)	Same as FE
Reporting of diagnosis, symptoms, or ever asthma	8	0.83 (0.73–0.94)	4.64 (7)	0.70	0 (0–68)	Same as FE
Adjustments						
No adjustment	7	0.92 (0.75–1.12)	6.06 (6)	0.42	1 (0–71)	0.92 (0.75–1.13)
Partial adjustment (mainly age and sex)	2	0.83 (0.62–1.12)	0 (1)	1.00	NA[a]	Same as FE
Comprehensive adjustment	7	0.85 (0.77–0.93)	7.59 (6)	0.27	21 (0–64)	0.84 (0.75–0.95)
Quality[d]						
Highest	3	0.82 (0.70–0.97)	0.85 (2)	0.65	0 (0–90)	Same as FE
Intermediary	10	0.88 (0.80–0.97)	10.43 (9)	0.32	14 (0–55)	0.89 (0.79–1.01)
Lowest	3	0.77 (0.59–1.01)	1.70 (2)	0.43	0 (0–90)	Same as FE

[a]Df must be $\geqslant 2$.
[b]The study-specific ORs used were based on the hierarchical assignment criterion applied in choosing the most valid estimate.
[c]The majority of studies considering wheezing relied on reporting for outcome ascertainment, hence no subgroup analysis was conducted.
[d]The highest quality studies had a score $\geqslant 1.4$, the intermediary had a score of 1.2–1.3 and the lowest had a score $\leqslant 1.1$.

By visual inspection of the Galbraith plot, no residual heterogeneity was detected as no studies lie outside the 95% limits (Figure 3). This can also be visually deduced from Figure 2 where the dashed line crosses the horizontal lines of all 16 individual studies, implying the absence of heterogeneity. There was some indication for publication bias as the funnel plot (Figure 4) showed slight departures from symmetry. This was particularly due to two large studies showing positive effects. However, the Egger's test for publication bias suggested absence of publication bias ($P = 0.73$).

Subgroup analyses

Table 4 presents pooled estimates from separate meta-analyses of the 16 studies across several subgroups defined by methodological characteristics. There was little evidence of heterogeneity with P values ranging from 0.09 to 1.00. In general, heterogeneity was more pronounced within studies that relied on current wheezing as an asthma definition. A subgroup analysis of 13 studies having either the highest or intermediary quality scores showed a similar association; the OR was 0.87 (0.80–0.94) with no evidence of heterogeneity ($Q = 11.80$, $P = 0.46$, $I^2 = 0\%$).

Discussion

The fact that the studies analysed did not all use a consistent measure of exposure or outcome provided the opportunity to evaluate this source of variability among studies. Some studies examined BCG vaccination, whereas others looked at tuberculin response or the size of the scar. Outcome measures included asthma and wheezing defined in a number of different ways. The association of exposure to BCG with

asthma was generally robust using different analysis strategies. Moreover, focussing on the most valid exposure indicator (BCG vaccination) strengthened the credibility of the results. The meta-analysis of 16 epidemiologic studies was in support of a protective effect of BCG vaccination against asthma development with an overall pooled OR of 0.86 (0.79–0.93).

Strengths and limitations

Our meta-analysis has several strengths. As a whole, we considered and fulfilled key requirements basic to performing and reporting a properly designed and valid meta-analysis.[63–65] We conducted a thorough review of the current literature, which enabled us to generate subgroup meta-analyses for three indicators of exposure to BCG and separately provided overall estimates for each indicator to assess the variability in association measures. A prior meta-analysis focussed solely on BCG vaccination per se,[8] whereas we considered two additional indicators of exposure in order to evaluate the variability in the association measures. We incorporated a methodological quality assessment using a comprehensive appraisal instrument that considered several epidemiological concepts including how subjects were selected, participation rates, reliability and validity of exposure and outcome measurements methods, accounting for confounders, as well as internal and external generalizability.[19] Consequently, the subgroup analysis restricted to higher quality studies could be regarded as providing a more conservative estimate of the summary OR.

We attempted to include all relevant studies, even those unpublished,[51] and irrespective of the language of publication to minimize a potential for selection bias. Although no indication of severe publication bias was found, the possibility however remains that some unpublished or un-indexed studies could have been missed. However, as seen from Figure 2, there was no tendency towards the null by the year of publication. In addition, the pooled OR for studies published prior to 2004 was 0.92 (0.80–1.06) with no evidence of heterogeneity ($Q = 3.51$, $P = 0.62$, $I^2 = 0\%$), whereas the pooled OR for publications from 2004 to 2009 was 0.82 (0.75–0.91), again with no evidence of heterogeneity ($Q = 8.97$, $P = 0.44$, $I^2 = 0\%$) (data not shown). Also, no linear effect of the year of publication was found upon carrying out a meta-regression ($P = 0.17$). Hence, we believe that publication bias was not a major concern affecting the conclusion reached in this meta-analysis.

Our findings have some limitations as well. Being a meta-analysis based upon summary statistics from mostly observational studies, it is prone to potential systematic errors inherent in the original studies (i.e. recall, selection and publication bias) and hence might compound these errors leading to faulty conclusions. These methodological drawbacks inherent to observational studies were acknowledged within the process of assigning quality scores. There was only one prospective study that did not however observe a significant relationship between BCG vaccination and asthma.[66] In the cross-sectional studies, the BCG exposure ascertainment methods were not considered to be the most valid as they were not based on records, but this aspect was accounted for in the quality score assignment process. Only one cross-sectional study was assigned a high-quality score.[61] This was due to reliance on official vaccination certificates, which are considered a valid method to ascertain BCG vaccination exposure. With respect to three case–control studies and seven retrospective cohort studies, recall bias was less of a concern because BCG vaccination was ascertained from records. Only one retrospective cohort study was assigned a low-quality score.[15] In this study, both exposure and outcome were based on subject reporting and no adjustment for potential confounders was done.

Although some studies adjusted for major important confounders,[24,51–55,61] others have not, and hence differential residual confounding by other factors cannot entirely be ruled out. Of note, these studies have generally adjusted for asthma risk factors. However, since the correlates of BCG vaccination are largely unknown, the potential confounders of the association between BCG vaccination and asthma are consequently also unknown. It could also be that different sets of correlates exist for each study population. However, comparing crude and adjusted estimates in the above mentioned eight studies was not suggestive of strong confounding effects (data not shown), and hence might not be a concern in studies with no or with partial adjustment for confounders.

Moreover, our analyses were limited in their ability to consider the timing of BCG vaccination, although it has been suggested as important with respect to asthma[67] and tuberculin reactions.[68] In most studies, the exact age at BCG vaccination was not mentioned, preventing us assessing the effect of BCG vaccinations at an early age versus later in childhood. However, for six studies with subjects vaccinated within their first year of life, a protective effect of early BCG vaccination with the occurrence of childhood asthma was found; the overall pooled OR was 0.86 with a 95% CI 0.79–0.94 ($Q = 7.52$, $P = 0.19$, $I^2 = 34\%$). This provides, in part, an indication that reverse causation was improbable in this situation and that a causal relationship can be envisaged between BCG vaccination and asthma prevention.

Our analyses were also limited in their ability to distinguish between prevalent and incident asthma cases, which precluded us from carrying out stratified analyses. We expect that there was a combination of both in each of the primary studies as no specific information that would indicate otherwise was provided, such as age at onset or first time diagnosis. One possible pitfall of being unable to distinguish prevalent from incident asthma cases is reverse

causation, which was an unlikely possibility as explained above. Another possible pitfall is the incidence–prevalence bias, the tendency to underestimate the strength of the association between BCG vaccination and asthma development, which would result in a more conservative estimate of the risk.[69]

Comparison with prior meta-analysis

Our findings contradict those reported in a prior meta-analysis where no association, predisposing or protective, was found between BCG vaccination and development of childhood asthma.[8] This discrepancy could be explained by several factors. To begin with, we have identified and included a relatively larger number of studies in our meta-analysis increasing the number of studies from 5 to 16. This allowed us to perform in-depth subgroup analyses according to several study characteristics. We also included those studies that were excluded from the prior meta-analysis such as cross-sectional studies[21,22,41,58–60] and studies that did not validate BCG vaccination events by medical records,[15,53] because we wanted to examine the differences and variability in results across various designs and exposure ascertainment methods. Of note, if we were to employ in our meta-analysis inclusion and exclusion criteria similar to those of the prior meta-analysis that included five studies, the OR based on eight studies that we identified as eligible, according to the FE model, would be 0.88 with a 95% CI: 0.78–0.99 ($Q = 9.55$, $P = 0.22$, $I^2 = 27\%$).

Immunological mechanism

The possible immunological mechanisms underlying the observed epidemiological associations between BCG vaccination and asthma are similar to those underlying the hygiene hypothesis.[70] According to this theory, exposure to microbes in early life may prime the immune system resulting in an up-regulation of T_H1 cells and a down-regulation of T_H2 lymphocytes. The diminished production of T_H2 cytokines thereby reduces the propensity to develop IgE-mediated atopic disorders.[71–73] Likewise, BCG vaccination may skew the cytokine microenvironment towards preferential selection of T_H1 cytokines and contribute to a decreased risk of T_H2-dependent atopic disease. The findings from several animal studies which demonstrated that BCG can suppress allergic and inflammatory responses,[74–78] allergic airway eosinophilia,[79–83] as well as the development of increased airway responsiveness,[81–85] support this hypothesis. Furthermore, a recent animal study has shown that neonatal BCG vaccination elicited long-term protection by inhibiting allergic airway inflammation in young and adult mice, and in the latter this was not mediated by the modulation of T_H1/T_H2 cytokine production.[86] The authors suggested that other mechanism(s) may underlie the long-lasting protection of BCG vaccination in aged mice, possibly related to regulatory T cells, which might play a crucial role in inhibiting inflammatory processes.[87,88]

Our meta-analysis focussed on the BCG vaccine as one particular example that induces a T_H1 type immune response. It is biologically plausible that other inducers of this type of immune response could have a similar asthma-preventive effect such as mycobacterial infections and other childhood vaccinations including pertussis vaccination. However, no evidence was found that infant vaccinations were related to the development of allergic diseases in a qualitative review[12] or in a meta-analysis limited to seven studies on the association between whole-cell pertussis vaccination and childhood asthma.[8] A systematic review of the relationship between mycobacterial infection and atopic disease revealed a high level of heterogeneity of studies.[7] Only three cross-sectional studies were in support of a protective effect of positive TST and atopy, whereas no association was found between BCG vaccination and asthma or atopy.

Conclusion

Overall, the results of this meta-analysis strengthen the epidemiological evidence in support of the immunological hypothesis that exposure to the BCG vaccine in early life may influence immune maturation and prevent asthma from developing, and hence might have far-reaching public health implications. Prospective clinical experiments, especially among predisposed and susceptible infants, will be informative regarding the benefits and risks of a simple preventive measure (BCG vaccination or similar immune modulators) that could reduce asthma prevalence and consequently the costs associated with its treatment and management. These studies are feasible to conduct especially in countries where the prevalence of tuberculosis is low and there are no BCG vaccination programs in effect. To our knowledge, Steenhuis and colleagues have initiated the first study on primary prevention of allergy in high-risk infants, investigating the administration of BCG at the age of 6 weeks in a randomized, placebo-controlled trial.[43] Results at 18 months of follow-up showed a trend towards less eczema in the BCG group compared with the placebo group, whereas no difference was observed in the prevalence of asthma between the two groups. However, a longer period of follow-up will be necessary to evaluate the potential preventive role of BCG vaccination in childhood asthma development.

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KEY MESSAGES

- The overall pooled OR of 16 epidemiological studies was 0.86 (95% CI 0.79–0.93), indicating a protective effect of BCG vaccination with the occurrence of asthma in childhood.
- The pooled OR from stratified analyses by several methodological covariates was consistent with the overall pooled OR.
- Exposure to the BCG vaccine in early life may influence immune maturation and prevent asthma development, and might have far-reaching public health implications.

References

[1] Garner R, Kohen D. Changes in the prevalence of asthma among Canadian children. *Health Rep* 2008;**19:**45–50.

[2] Glaxo Wellcome Inc. *Asthma in Canada A landmark Survey™*. Mississauga, Ontario: Glaxo Wellcome Inc., 2000.

[3] The National Asthma Control Task Force. The Prevention and management of asthma in Canada: a major challenge now and in the future. Ottawa: Health Canada, 2000.

[4] Millar WJ, Hill GB. Childhood asthma. *Health Rep* 1998;**10**:9–21 (ENG); 9–22 (FRE).

[5] Robinson DS, Hamid Q, Ying S *et al*. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;**326:**298–304.

[6] Kon OM, Kay AB. T cells and chronic asthma. *Int Arch Allergy Immunol* 1999;**118:**133–35.

[7] Obihara CC, Bollen CW, Beyers N, Kimpen JL. Mycobacterial infection and atopy in childhood: a systematic review. *Pediatr Allergy Immunol* 2007;**18:** 551–59.

[8] Balicer RD, Grotto I, Mimouni M, Mimouni D. Is childhood vaccination associated with asthma? A meta-analysis of observational studies. *Pediatrics* 2007;**120:** e1269–77.

[9] Liu J, Tran V, Leung AS, Alexander DC, Zhu B. BCG vaccines: their mechanisms of attenuation and impact on safety and protective efficacy. *Hum Vaccin* 2009; **5:**70–78.

[10] Hawgood BJ. Albert Calmette (1863–1933) and Camille Guerin (1872–1961): the C and G of BCG vaccine. *J Med Biogr* 2007;**15:**139–46.

[11] Krishna MT, Salvi SS. Could administration of bacille Calmette-Guerin vaccination at birth protect from the development of asthma and allergic diseases in the western world? Has this question been adequately investigated? *Pediatr Allergy Immunol* 2002;**13:**172–76.

[12] Koppen S, de Groot R, Neijens HJ, Nagelkerke N, van Eden W, Rumke HC. No epidemiological evidence for infant vaccinations to cause allergic disease. *Vaccine* 2004;**22:**3375–85.

[13] Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;**275:**77–79.

[14] Samuel G. Childhood BCG vaccination and the risk of asthma in adults. M.Sc. Thesis. McGill University, 2004.

[15] Benke G, Abramson M, Raven J, Thien FC, Walters EH. Asthma and vaccination history in a young adult cohort. *Aust N Z J Public Health* 2004;**28:**336–38.

[16] Mommers M, Weishoff-Houben M, Swaen GM *et al*. Infant immunization and the occurrence of atopic disease in Dutch and German children: a nested case-control study. *Pediatr Pulmonol* 2004;**38:**329–34.

[17] Menzies D. What does tuberculin reactivity after bacille Calmette-Guerin vaccination tell us? *Clin Infect Dis* 2000; **31:**S71–74.

[18] Hall CB, Wakefield D, Rowe TM, Carlisle PS, Cloutier MM. Diagnosing pediatric asthma: validating the Easy Breathing Survey. *J Pediatr* 2001;**139:**267–72.

[19] Genaidy AM, Lemasters GK, Lockey J *et al*. An epidemiological appraisal instrument – a tool for evaluation of epidemiological studies. *Ergonomics* 2007;**50:**920–60.

[20] Alm JS, Lilja G, Pershagen G, Scheynius A. Early BCG vaccination and development of atopy. *Lancet* 1997;**350:** 400–3.

[21] Pahari A, Welch S, Lingam S. BCG, tuberculin skin-test results and asthma prevalence in school children in North London. *Indian Pediatr* 2002;**39:**254–58.

[22] Bibakis I, Zekveld C, Dimitroulis I *et al*. Childhood atopy and allergic disease and skin test responses to environmental mycobacteria in rural Crete: a cross-sectional survey. *Clin Exp Allergy* 2005;**35:**624–69.

[23] Soysal A, Bahceciler N, Barlan I, Bakir M. Lack of an inverse association between tuberculosis infection and atopy: By T-cell-based immune assay (RD1-ELISpot). *Pediatr Allergy Immunol* 2008;**19:**709–15.

[24] Marks GB, Ng K, Zhou J *et al*. The effect of neonatal BCG vaccination on atopy and asthma at age 7 to 14 years: an historical cohort study in a community with a very low prevalence of tuberculosis infection and a high prevalence of atopic disease. *J Allergy Clin Immunol* 2003;**111:**541–59.

[25] Queiroz RM, Sarinho SW, Sarinho E, Ximenes R. Relationship between BCG scar size and asthma in children? *Indian Pediatrics* 2004;**41:**916–21.

[26] Gruber C, Kulig M, Bergmann R, Guggenmoos-Holzmann I, Wahn U. Delayed hypersensitivity to tuberculin, total immunoglobulin E, specific sensitization, and

atopic manifestation in longitudinally followed early Bacille Calmette-Guerin-vaccinated and nonvaccinated children. *Pediatrics* 2001;**107:**E36.

[27] Annus T, Montgomery SM, Riikjarv MA, Bjorksten B. Atopic disorders among Estonian schoolchildren in relation to tuberculin reactivity and the age at BCG vaccination. *Allergy* 2004;**59:**1068–73.

[28] Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;**332:**133–38.

[29] Klinnert MD, Nelson HS, Price MR, Adinoff AD, Leung DY, Mrazek DA. Onset and persistence of childhood asthma: predictors from infancy. *Pediatrics* 2001; **108:**E69.

[30] Hardy RJ, Thompson SG. Detecting and describing heterogeneity in meta-analysis. *Stat Med* 1998; **17:**841–56.

[31] DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;**7:**177–88.

[32] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959;**22:**719–48.

[33] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;**21:**1539–58.

[34] Xu H, Platt RW, Luo ZC, Wei S, Fraser WD. Exploring heterogeneity in meta-analyses: needs, resources and challenges. *Paediatr Perinat Epidemiol* 2008;**22 (Suppl 1):** 18–28.

[35] Jackson D. The power of the standard test for the presence of heterogeneity in meta-analysis. *Stat Med* 2006;**25:**2688–99.

[36] Galbraith RF. A note on graphical presentation of estimated odds ratios from several clinical trials. *Stat Med* 1988;**7:**889–94.

[37] Tobias A. sbe26: Assessing the influence of a single study in meta-analysis. *Stata Tech Bull* 1999;**47:**15–17.

[38] Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**315:**629–34.

[39] Sterne J, Bradburn M, Egger M. Meta-analysis in Stata. In: Egger M, Smith G, Altman D (eds). *Systematic Reviews in Health Care: Meta-analysis in Context*. London: BMJ Publishing Group, 2001, pp. 347–69.

[40] Castanon LA, Perez Lopez J, Rosas Vargas MA, Del Rio Navarro BE, Sienra Monge JJL. [Reaction to PPD and its relationship with allergic diseases in children vaccinated with BCG at birth]. *Revista Alergia México* 2003;**50:**48–53.

[41] Yoneyama H, Suzuki M, Fujii K, Odajima Y. The effect of DPT and BCG vaccinations on atopic disorders. *Arerugi* 2000;**49:**585–92.

[42] Ma Y, Li XF, Zhao J, Wong GW, Zhou CS, Chen YZ. Relationships between the diameters of Bacille Calmette-Guerin scars and asthma, atopy in urban and rural Beijing children. *Zhonghua Jie He He Hu Xi Za Zhi* 2003;**26:**526–30.

[43] Steenhuis TJ, van Aalderen WM, Bloksma N *et al*. Bacille-Calmette-Guerin vaccination and the development of allergic disease in children: a randomized, prospective, single-blind study. *Clin Exp Allergy* 2008;**38:** 79–85.

[44] Townley RG, Barlan IB, Patino C *et al*. The effect of BCG vaccine at birth on the development of atopy or allergic disease in young children. *Ann Allergy Asthma Immunol* 2004;**92:**350–55.

[45] Bager P, Rostgaard K, Nielsen NM, Melbye M, Westergaard T. Age at bacille Calmette-Guerin vaccination and risk of allergy and asthma. *Clin Exp Allergy* 2003;**33:**1512–17.

[46] Martignon G, Oryszczyn MP, Annesi-Maesano I. Does childhood immunization against infectious diseases protect from the development of atopic disease? *Pediatr Allergy Immunol* 2005;**16:**193–200.

[47] Yilmaz M, Bingol G, Altintas D, Kendirli SG. Correlation between atopic diseases and tuberculin responses. *Allergy* 2000;**55:**664–67.

[48] Ozmen S, Tomac N, Uysal A, Arslan Z, Kuyucu N, Yoney A. Tuberculin responses in children with allergic diseases. *Allergy* 2002;**57:**1059–62.

[49] Strannegard IL, Larsson LO, Wennergren G, Strannegard O. Prevalence of allergy in children in relation to prior BCG vaccination and infection with atypical mycobacteria. *Allergy* 1998;**53:**249–54.

[50] Ahmadiafshar A, Parchegani MR, Moosavinasab N, Koosha A. A Study of Relation between BCG Scar and Atopy in Schoolchildren of Zanjan City. *Iran J Allergy Asthma Immunol* 2005;**4:**185–88.

[51] Samuel G. *Childhood BCG vaccination and the risk of asthma in adults*. M.Sc Thesis: McGill University, 2004.

[52] Gruber C, Meinlschmidt G, Bergmann R, Wahn U, Stark K. Is early BCG vaccination associated with less atopic disease? An epidemiological study in German preschool children with different ethnic backgrounds. *Pediatr Allergy Immunol* 2002;**13:**177–81.

[53] Garcia-Marcos L, Suarez-Varela MM, Canflanca IM *et al*. BCG immunization at birth and atopic diseases in a homogeneous population of Spanish schoolchildren. *Int Arch Allergy Immunol* 2005;**137:**303–39.

[54] Linehan MF, Frank TL, Hazell ML *et al*. Is the prevalence of wheeze in children altered by neonatal BCG vaccination? *J Allergy Clin Immunol* 2007;**119:**1079–85.

[55] Miyake Y, Arakawa M, Tanaka K, Sasaki S, Ohya Y. Tuberculin reactivity and allergic disorders in schoolchildren, Okinawa, Japan. *Clin Exp Allergy* 2008; **38:**486–92.

[56] Sarinho E, Schor D, Veloso M, Lima M. BCG scar diameter and asthma: a case-control study. *J Allergy Clin Immunol* 2000;**106:**1199–200.

[57] Wickens K, Crane J, Kemp T *et al*. A case-control study of risk factors for asthma in New Zealand children. *Aust N Z J Public Health* 2001;**25:**44–49.

[58] Wong GW, Hui DS, Tam CM *et al*. Asthma, atopy and tuberculin responses in Chinese schoolchildren in Hong Kong. *Thorax* 2001;**56:**770–73.

[59] da Cunha SS, Cruz AA, Dourado I, Barreto ML, Ferreira LD, Rodrigues LC. Lower prevalence of reported asthma in adolescents with symptoms of rhinitis that received neonatal BCG. *Allergy* 2004;**59:**857–62.

[60] Obihara CC, Kimpen JL, Gie RP *et al*. Mycobacterium tuberculosis infection may protect against allergy in a tuberculosis endemic area. *Clin Exp Allergy* 2006;**36:**70–76.

[61] Mohrenschlager M, Haberl VM, Kramer U, Behrendt H, Ring J. Early BCG and pertussis vaccination and atopic

diseases in 5- to 7-year-old preschool children from Augsburg, Germany: results from the MIRIAM study. *Pediatr Allergy Immunol* 2007;**18:**5–9.

[62] Soysal A, Bahceciler N, Barlan I, Bakir M. Lack of an inverse association between tuberculosis infection and atopy: By T-cell-based immune assay (RD1-ELISpot). *Pediatr Allergy Immunol* 2008;**19:**709–15.

[63] Walker E, Hernandez AV, Kattan MW. Meta-analysis: Its strengths and limitations. *Cleve Clin J Med* 2008;**75:** 431–39.

[64] Berman NG, Parker RA. Meta-analysis: neither quick nor easy. *BMC Med Res Methodol* 2002;**2:**10.

[65] Stroup DF, Berlin JA, Morton SC *et al*. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000;**283:**2008–12.

[66] Grüber C, Kulig M, Bergmann R, Guggenmoos-Holzmann I, Wahn U. Delayed hypersensitivity to tuberculin, total immunoglobulin E, specific sensitization, and atopic manifestation in longitudinally followed early Bacille Calmette-Guerin-vaccinated and nonvaccinated children. *Pediatrics* 2001;**107:**E36.

[67] Barlan I, Bahceciler NN, Akdis M, Akdis CA. Bacillus Calmette-Guerin, Mycobacterium bovis, as an immunomodulator in atopic diseases. *Immunol Allergy Clin North Am* 2006;**26:**365–77, ix.

[68] Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis* 2006;**10:**1192–204.

[69] Szklo M, Nieto FJ. *Epidemiology Beyond the Basics*. 2nd edn. Massachusetts, USA: Jones and Bartlett Publishers, Inc., 2007.

[70] Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;**299:**1259–60.

[71] Vandenbulcke L, Bachert C, Van Cauwenberge P, Claeys S. The innate immune system and its role in allergic disorders. *Int Arch Allergy Immunol* 2006;**139:** 159–65.

[72] Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology* 2004;**112:** 352–63.

[73] Kemp A, Bjorksten B. Immune deviation and the hygiene hypothesis: a review of the epidemiological evidence. *Pediatr Allergy Immunol* 2003;**14:**74–80.

[74] Yang X, Fan Y, Wang S *et al*. Mycobacterial infection inhibits established allergic inflammatory responses via alteration of cytokine production and vascular cell adhesion molecule-1 expression. *Immunology* 2002;**105:**336–43.

[75] Major T, Wohlleben G, Reibetanz B, Erb KJ. Application of heat killed Mycobacterium bovis-BCG into the lung inhibits the development of allergen-induced Th2 responses. *Vaccine* 2002;**20:**1532–40.

[76] Tukenmez F, Bahceciler NN, Barlan IB, Basaran MM. Effect of pre-immunization by killed *Mycobacterium bovis* and *vaccae* on immunoglobulin E response in ovalbumin-sensitized newborn mice. *Pediatr Allergy Immunol* 1999;**10:** 107–11.

[77] Su YC, Peng HJ, Wang SR, Han SH, Tsai JJ. Effects of BCG on ovalbumin-induced bronchial hyperreactivity in a guinea pig asthma model. *J Microbiol Immunol Infect* 2001; **34:**25–34.

[78] Smit JJ, Van Loveren H, Hoekstra MO, Schijf MA, Folkerts G, Nijkamp FP. Mycobacterium vaccae administration during allergen sensitization or challenge suppresses asthmatic features. *Clin Exp Allergy* 2003;**33:** 1083–89.

[79] Erb KJ, Holloway JW, Sobeck A, Moll H, Le Gros G. Infection of mice with Mycobacterium bovis-Bacillus Calmette-Guerin (BCG) suppresses allergen-induced airway eosinophilia. *J Exp Med* 1998;**187:**561–69.

[80] Koh YI, Choi IS, Park SC, Kang KW. BCG infection during pre-sensitization or even post-sensitization inhibits airway sensitivity in an animal model of allergic asthma. *J Korean Med Sci* 2000;**15:**265–72.

[81] Hopfenspirger MT, Parr SK, Hopp RJ, Townley RG, Agrawal DK. Mycobacterial antigens attenuate late phase response, airway hyperresponsiveness, and bronchoalveolar lavage eosinophilia in a mouse model of bronchial asthma. *Int Immunopharmacol* 2001;**1:**1743–51.

[82] Riffo-Vasquez Y, Spina D, Page C *et al*. Effect of *Mycobacterium tuberculosis* chaperonins on bronchial eosinophilia and hyper-responsiveness in a murine model of allergic inflammation. *Clin Exp Allergy* 2004;**34:**712–19.

[83] Choi IS, Lin XH, Koh YA, Koh YI, Lee HC. Strain-dependent suppressive effects of BCG vaccination on asthmatic reactions in BALB/c mice. *Ann Allergy Asthma Immunol* 2005;**95:**571–78.

[84] Herz U, Gerhold K, Gruber C *et al*. BCG infection suppresses allergic sensitization and development of increased airway reactivity in an animal model. *J Allergy Clin Immunol* 1998;**102:**867–74.

[85] Nahori MA, Lagranderie M, Lefort J *et al*. Effects of Mycobacterium bovis BCG on the development of allergic inflammation and bronchial hyperresponsiveness in hyper-IgE BP2 mice vaccinated as newborns. *Vaccine* 2001;**19:**1484–95.

[86] Shen H, Huang H, Wang J *et al*. Neonatal vaccination with Bacillus Calmette-Guerin elicits long-term protection in mouse-allergic responses. *Allergy* 2008;**63:**555–63.

[87] Taams LS, Palmer DB, Akbar AN, Robinson DS, Brown Z, Hawrylowicz CM. Regulatory T cells in human disease and their potential for therapeutic manipulation. *Immunology* 2006;**118:**1–9.

[88] Wing K, Fehervari Z, Sakaguchi S. Emerging possibilities in the development and function of regulatory T cells. *Int Immunol* 2006;**18:**991–1000.

Appendix 1 Characteristics of 10 studies excluded from the systematic review and meta-analysis of the association between BCG exposure and childhood asthma

References	Age (years)	Exposure indicators	Exposure assessment	Age at exposure	No. exposed (diameter size)	No. unexposed (diameter size)	Outcome measures
Steenhuis *et al.*[43]	0–1.5	BCG vaccination	BCG administration	6 weeks	61	54	Clinical evidence and parental reporting of symptoms indicative of asthma, allergic rhinitis, eczema and food allergy
Townley *et al.*[44]	0–2	Tuberculin response	TST	9–12 months (vaccinated at birth)	350 ($\geqslant$5 mm)	305 (<3 mm)	Parental reporting of allergic history: wheezing, asthma, rhinitis, eczema
Bager *et al.*[45]	21–46	BCG vaccination	School records	0–15 years	1653	303	Subject reporting of asthma and allergic rhinitis, serological determination of atopic status
Martignon *et al.*[46]	12–15	BCG vaccination	Personal pediatric records	<6 months	647	24	Subject reporting of at least one atopic disease (asthma, allergic rhinitis including rhinoconjunctivitis and hay fever, eczema)
Yilmaz *et al.*[47]	2.5–16	Tuberculin response	TST	2.5–16 years (vaccinated at birth, 6 and 12 years)	272 ($\geqslant$10 mm)	464 (<10 mm)	Clinical evidence and history of allergic diseases (rhinitis, asthma, atopic dermatitis)
Ozmen *et al.*[48]	1–14	BCG vaccination	Scar presence	1–14 years	125	81	Parental and clinical assessment of allergic diseases (asthma, rhinitis, atopic eczema, urticaria)
		Tuberculin response	TST				
Strannegard *et al.*[49]	4–5, 7–8, 8–9	BCG vaccination	Questionnaire	$\leqslant$1 year	294	6203	Parental reporting of allergy (atopic dermatitis, allergic rhinoconjunctivitis, asthma)
			Presence of scar	4–5, 7–8 and 8–9 years			
		Tuberculin response	TST	4–5, 7–8 and 8–9 years	924 ($\geqslant$3 mm)	4880 (<3 mm)	
Castanon *et al.*[40]	2–7	Tuberculin response	TST	2–7 years (vaccinated at birth)	5 (>10 mm)	45 (<10 mm)	Diagnosed asthma, rhinitis, both
Ma *et al.*[42]	13–14	Scar diameter	Measurement of scar size	At birth			Subject reporting of asthma wheeze, hay fever ever, rash, eczema, allergic rhinitis, atopy
Ahmadiafshar *et al.*[50]	11–15	Scar diameter	Measurement of scar size	11–15 years	711 (>5 mm)	228 ($\leqslant$5 mm)	Subject reporting of asthma, rhinitis, atopic dermatitis

BCG: Bacillus Calmette-Guérin; TST: Tuberculin Skin Test.