

Original article

Lower prevalence of reported asthma in adolescents with symptoms of rhinitis that received neonatal BCG

Background: BCG is a vaccine used against tuberculosis and leprosy and is an immunostimulant that primes T_H1 lymphocytes to produce cytokines that antagonize atopy both in animal models and in man. Considering that atopy is the main risk factor for asthma, one can hypothesize that vaccination inducing T_H1 responses, such as BCG, can be protective against asthma.

Methods: Objective: To estimate the association between neonatal BCG vaccination and prevalence of asthma among adolescents. Study design: Cross-sectional study with schoolchildren aged 12–16 years. The presence of a scar compatible with BCG was used as a surrogate of neonatal vaccination. A self-administered structured questionnaire was prepared based on that used by the International Study of Asthma and Allergies in Childhood. The prevalence of asthma was categorized according to the report of lifetime wheeze, lifetime asthma, lifetime asthma among those referring allergy and among those referring allergy and sneezing.

Results: Neonatal BCG vaccination was not associated with the overall prevalence of reported wheezing or asthma. However, in the subgroup reporting current allergy and sneezing, neonatal BCG was associated with a 37% reduction of prevalence of lifetime asthma.

Conclusions: In the population we surveyed, neonatal BCG scar was associated with a reduction in the risk of asthma only in individuals with a past history suggestive of allergic rhinitis.

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BCG is a vaccine used against tuberculosis and leprosy and is an immunostimulant that primes T_H1 lymphocytes to produce cytokines that antagonize atopy both in animal models (1, 2) and in man. Considering that atopy is the main risk factor for asthma, one can hypothesize that vaccination inducing T_H1 responses can be protective against asthma (3). However, there are conflicting data about the effect of BCG vaccination on asthma, and regarding the inter-relationship between asthma and atopy. Alm et al. (4) and Anderson et al. (5) found no association between atopic disorders and BCG vaccination. On the contrary, Marks et al. reported an association between neonatal BCG and reduction in the prevalence of asthma among those with family history of rhinitis or eczema (6). Strannegard et al. and Aaby et al. reported association between BCG vaccination and lower rates of atopic disorders (7, 8). Tuberculin response is a marker of T_H1 -mediated acquired immunity, but there is conflicting evidence on the association between tuberculin reactions, and allergy and asthma: Shirakawa et al. (9) showed that a positive tuberculin response was associated with lower occurrence of asthma and allergy, but his finding was not reproduced in other studies (10–13). Furthermore,

there is no conclusive evidence of the efficacy of microbial products against allergy (14). Therefore, the association between BCG and asthma remains matter of debate.

An increase in the prevalence of respiratory diseases related to atopy has been described in the western world (15), and any protective effect of BCG against asthma would be of public health interest, given the widespread use of this vaccine of low cost and high safety. This paper describes a cross-sectional study aimed to estimate the association between neonatal BCG vaccination and the prevalence of asthma in adolescents in Brazil. Our main hypothesis was that adolescents who had received neonatal BCG have lower prevalence of asthma.

Methods

Study design, population and sampling

This was a cross sectional study of schoolchildren, nested in the study population of a large controlled trial currently running in Brazil [details have been published elsewhere (16)]. Briefly, the trial aims to evaluate the effectiveness of BCG vaccine given

to schoolchildren with a high coverage of neonatal BCG. The lyophilized BCG used in Brazil is produced by a private non-profitable foundation using Moreau strain. The trial bridges two study sites but this asthma study was conducted in the population of the city of Salvador only. Salvador has 2.5 million inhabitants and is located in the coast with a tropical climate and high humidity. A prevalence of 10.0% of self-reported asthma among schoolchildren aged 13–14 years was found in a recent survey (17).

The sample size was estimated to detect a reduction of 50% in the prevalence of lifetime asthma in children with neonatal BCG vaccination, with an alpha level of 5%, assuming 10% of lifetime prevalence of asthma among those not vaccinated. These parameters required a sample size of 620 participants in each group (with and without neonatal BCG vaccination) for a study power of 90%. First, a sample of the schools enrolled in the original trial and located in the central area of the city was selected for convenience reasons. Secondly, students were selected randomly from a list of those enrolled in the selected schools. Data on BCG scar (collected earlier for the main trial) and on date of birth were extracted from the trial database. The inclusion criteria were having either no scar or one scar only; and being aged 12–16 years in 1999 (when this study was conducted). Children who did not meet these criteria were excluded.

Data collection

BCG vaccination: neonatal and at school age. The presence of a scar compatible with BCG was used as a surrogate of neonatal vaccination in the trial and in our study. In Brazil, most children aged 0–4 years are vaccinated with BCG, and a second dose was given to nearly half of schoolchildren aged 7–14 years attending public schools in Salvador, as part of the protocol of the randomized controlled trial of BCG revaccination effectiveness. Vaccination is through intradermal injection (0.1 ml) in the deltoid region of the right arm. In the trial and in the routine neonatal vaccination, the vaccine used is the lyophilized BCG containing the Moreaux RIO strain (stands for *Rio de Janeiro*). Data on BCG scar was ascertained during the recruitment phase of the trial in 1996–1997 by trained health workers who visited the schools, and are available from the trial database. Validation of the scar as a marker of BCG vaccination in the trial population in Salvador has been published elsewhere (18). Briefly, high inter-observer agreement (Kappa = 0.84, $P < 0.001$) was found, as well as a sensitivity of 98% and specificity of 92% considering as gold standard the agreement between parental information and vaccine card. The database also contained information on those children who received BCG at school age as part of the trial.

Asthma and allergy. A structured questionnaire was prepared based on that used by the International Study of Asthma and Allergies in Childhood (ISAAC) Study. Our questionnaire had three parts. The first part gave instructions on completion and collected data on identification. The second part, on asthma, was almost identical to the ISAAC questionnaire and comprised eight questions (19). It differed from the ISAAC questionnaire in a critical way. The most common word in Brazilian Portuguese to express wheezing is 'chiado' or 'piado', and these were used in the ISAAC study. However, a committee of local paediatricians consulted for this study suggested using the expression 'cansaço' (literally 'being tired' but also meaning 'breathlessness'), which is commonly used by the population of Salvador. This expression was added to 'chiado' or 'piado' in questions where the English version used 'wheezing', but not when the English and Portuguese versions used the term 'asthma' (Question 6 in ISAAC questionnaire). The third part of the

questionnaire (not based on the ISAAC questionnaire) collected information on allergy based on self-reporting of current allergy and whether this was accompanied by sneezing and/or skin manifestations.

Questionnaires were self administered. They were distributed to students by trained field workers who also gave instructions on how to complete the questionnaire. Field workers visited each school twice, and the second time aimed to contact the students absent during the first visit. Questionnaires were completed from June to August 1999, 2 years after the trial vaccination phase. In the original trial, the geographical areas of the city were categorized in four socio-economic status based on average income per household obtained from the census of 1990. This categorization was used to classify the schoolchildren in to four socio-economic statuses according to schools they were attending or living.

Analysis

Categories for outcome were created combining different variables from the questionnaire. The outcome *lifetime wheezing* corresponded to question 1 of the ISAAC questionnaire (have you ever had wheezing or whistling in the chest at any time in the past?). The proportion of positive answers corresponds to the lifetime cumulative prevalence of wheezing. The outcome *lifetime asthma* corresponded to question 6 in the ISAAC questionnaire (have you ever had asthma at any time in the past?) and corresponds to lifetime cumulative prevalence of asthma. The prevalence of this last outcome was calculated for the total population and separately for two sub-groups: those reporting allergy and for those reporting allergy and sneezing.

All the analysis was carried out excluding those with missing data (complete data analysis). Association between categorical variables was estimated using odds ratios (ORs) and chi square tests. The study population was selected through cluster sampling, and effect of clustering was taken into account in the analysis (20). The chi square test was adjusted by cluster according to Rao (21), but the point estimates for the prevalence rates were not. The estimates of the ORs between each defined outcome and BCG scar were obtained through logistic regression with random-effects (20). All calculations were performed in STATA (22).

Results

The questionnaire was applied to 1612 schoolchildren in 36 different schools, of which 1437 (89.1%) during the first visit and 175 (10.9%) in a second visit. Among all individuals, 523 (32.4%) had no neonatal BCG scar, and among these 523, 220 had received BCG at school during the trial. The remaining 303 were never vaccinated. Among the 1089 (67.6%) with neonatal BCG scar, 504 were revaccinated at school by the trial and 585 were not. Twenty-three individuals did not answer the questions

Table 1. Characteristics of the study population by neonatal BCG scar

Characteristic	Presence of BCG scar (n, %)		Chi square adjusted for cluster
	Yes	No	
Total population	1089 (67.6)	523 (32.4)	
Sex			
Male	535 (71.0)	218 (29.0)	$\chi^2 = 4.4$
Female	554 (64.5)	305 (35.5)	$P = 0.04$
Current maternal smoking			
Yes	109 (60.9)	70 (39.1)	$\chi^2 = 5.9$
No	721 (70.3)	304 (29.7)	$P = 0.02$
Missing data		408	
Current allergy with sneezing			
Yes	397 (71.3)	160 (28.7)	$\chi^2 = 7.2$
No	669 (65.3)	355 (34.7)	$P = 0.01$
Missing data		31	
Socio-economic status			
Richest	43 (72.9)	16 (27.1)	$\chi^2 = 2.1$
Middle 1	60 (69.0)	27 (31.0)	
Middle 2	420 (70.6)	175 (29.4)	
Poorest	565 (64.9)	306 (35.1)	
Number of householders			
0-3	141 (71.6)	56 (28.4)	$\chi^2 = 3.06$
4	252 (72.4)	96 (27.6)	
5	264 (61.0)	119 (39.0)	
6	190 (67.4)	92 (32.6)	
7	91 (57.2)	68 (42.8)	
8 or more	132 (60.3)	87 (39.7)	
Missing data		24	
Parental asthma			
Yes	570 (66.4)	288 (33.6)	$\chi^2 = 1.11$
No	223 (69.0)	100 (31.0)	$P = 0.30$
Missing data		431	
Pet ownership			
Yes	731 (66.6)	366 (33.4)	$\chi^2 = 1.44$
No	349 (69.8)	151 (30.2)	$P = 0.24$
Missing data		15	
Current allergy skin manifestations			
Yes	100 (64.1)	56 (35.9)	$\chi^2 = 3.85$
No	537 (70.9)	220 (29.1)	$P = 0.07$
Missing data		699	

about wheezing or asthma; they were similar to respondents regarding age, sex and socio-economic status (data not shown).

The prevalence of BCG scar according to several study variables is shown in Table 1. A greater proportion of individuals with neonatal BCG scar was found among males (71.0% vs. 64.5%), among those without history of current maternal smoking (70.3% vs 60.9%), among those reporting current allergy with sneezing (71.3% vs 65.3%) and among those who lived with fewer householders (all statistically significant results at an alpha level of 5%). The prevalence of BCG scar was not statistically different according to socio-economic status, parental asthma, current paternal smoking, current allergy with skin lesion, pet ownership and BCG vaccination at school during the trial (yes = 69.6% vs no = 65.9%) (data not shown). The mean age was the same among those with and without BCG scar: mean 14.0 and SD = 0.9, for both sexes.

Table 2 presents the prevalence of the outcomes according to neonatal BCG scar. Thirty-seven per cent of the population reported *lifetime wheezing*, but only 8.9% reported *lifetime asthma*. The prevalence of lifetime asthma among those with allergy and sneezing was lower among those with neonatal BCG scar than among those without it (14.7% vs 23.4%, $P = 0.03$). The prevalence of wheezing after exercise in the last 12 months was lower among those with neonatal BCG (30.4% vs 34.9%, $P = 0.04$). There were no other significant results.

The overall prevalence of reported allergy was 57.7%, mainly allergy with sneezing (35.1%) and the prevalence of allergy with skin manifestations was 9.8% (data not shown). The estimated prevalence of *lifetime asthma* among the subjects who received recent BCG revaccination was 9.3% (59 of 707) whereas among those who did not receive recent BCG revaccination was 8.4% (81 of 869) ($P = 0.33$). Likewise, the prevalence of 'wheezing in the last 12 months' was 21.4% (168 of 883) in recently

Table 2. Comparison of the prevalence (%) of the outcomes between those with BCG scar and those with no BCG scar

Outcome	Presence of BCG scar						χ^2 ; <i>P</i> -value adjusted for cluster*
	Total		Yes		No		
	%	No. of responders	%	No. of responders	%	No. of responders	
Lifetime wheezing	36.7	1601	36.7	1083	36.7	518	0.99
Episodes of wheezing in the last 12 months among those with lifetime wheezing	47.2	572	46.5	389	48.6	183	0.54
Sleep disturbed in the last 12 months among those with lifetime wheezing	29.9	569	29.6	388	30.4	181	0.93
Lifetime asthma							
In the total population	8.9	1576	8.4	1064	10.0	512	0.32
Among those with allergy	13.0	886	11.7	617	16.0	269	0.11
Among those with allergy and sneezing	17.2	546	14.7	388	23.4	158	0.03
Wheezing after exercise last 12 months	31.9	1597	30.4	1078	34.9	519	0.04
Cough at night last 12 months	32.6	1600	32.7	1081	32.4	519	0.90

* Chi square test compares the proportion of each outcome between those with and without BCG scar.

Table 3. Adjusted odds ratios for the association between neonatal BCG scar and outcomes

Results for complete data-set adjusted for parental asthma	Outcomes			
	Lifetime asthma			
	Lifetime wheezing	Total population	Among those with allergy	Among those with allergy and sneezing
Odds ratio (BCG and outcome)	0.99	0.83	0.69	0.58
95% confidence interval	0.79–1.27	0.58–1.20	0.45–1.04	0.36–0.92
Number of individuals	1574	1550	869	536

revaccinated subjects and 19.03% (154 of 719) among those not revaccinated ($P = 0.40$) (data not shown).

Current maternal smoking was significantly associated with *lifetime asthma* in the total population (OR = 1.9; 95% CI: 1.1–3.1). Current paternal smoking was not significantly associated with any study variable. Having a history of parent with asthma was significantly associated with lifetime wheezing (OR = 3.5; 95% CI: 2.5–4.7), with lifetime asthma in the total population (OR = 3.1; 95% CI: 1.7–5.6) and in those with reported allergy (OR = 2.4; 95% CI: 1.2–4.6). Reported allergy with sneezing was associated with lifetime wheezing (OR = 2.3; 95% CI: 1.7–3.1) and lifetime asthma in the total population (OR = 3.1; 95% CI: 1.9–5.0). The prevalence of reported lifetime wheezing or asthma were not statistically significant different according to the existence of animal at home, sex, age, number of householders, socio-economic status and BCG vaccination during the trial (data not shown).

In the logistic regression using random effects with complete data-set, the ORs between BCG scar and each of the four outcomes did not change in comparison with the simpler model including only BCG scar and each outcome, and thus we consider that confounding was not present in this data-set. Therefore, no adjustment is necessary. The association between lifetime asthma and neonatal BCG among those with allergy and sneezing, adjusted for parental asthma was OR = 0.58 (95% CI: 0.36–0.92) (Table 3).

Discussion

Main finding

In Brazilian adolescents, neonatal BCG vaccination as indicated by the presence of BCG scar, was not associated with the overall prevalence of reported wheezing or asthma. However, in the subgroup reporting current allergy and sneezing, neonatal BCG was associated with a 37% reduction of prevalence of lifetime asthma. School age vaccination had no effect on the prevalence of reported wheezing or asthma.

Weakness and strength of the study

The study has some limitations. We estimated the outcomes based on a questionnaire, without an objective diagnostic procedure. A history of repeated wheezing episodes is a very sensitive, although not a very specific indicator of asthma (19). The introduction of a new term (cansaço) in the questionnaire was not validated, and because it can also mean tiredness, it is likely to have decreased the specificity of the term wheezing. Information on BCG vaccination was based on scar reading. This is unlikely to be a weakness of the study as it has been shown to be very accurate in this population (18). Lack of precision would result in nondifferential misclassification of exposure or outcome and consequently in underestimation of the association, but would not cause a positive finding. In our survey the term ‘current allergy with sneezing’, as a proxy of atopy (rhinitis), was self reported and was not validated. However, atopy is associated with asthma (23), and the association between asthma and the report of current allergy with sneezing in our findings suggests that we were able to identify individuals with atopy correctly. In our region of Brazil there is no pollinosis but only rhinitis related to perennial allergens. In this situation conjunctival manifestations are not so common.

Interpretation of the results

Other studies did not show a consistent association between BCG vaccination with low occurrence of asthma (24, 25). We found an association only in children with allergy and sneezing. Why did we find a protective effect of BCG against asthma in children with allergy and sneezing, but no BCG protection against asthma alone? We suggest three possible reasons. First, this may be a chance finding. Secondly, the restriction of the analysis of the subgroup of those referring allergy and sneezing might have restored enough specificity to both diagnoses for us to detect a protective effect. In other words, specificity may be lower for asthma alone and allergy and sneezing alone, than for the two diagnoses combined. Thirdly, maybe the effect of BCG is stronger when asthma has an atopic component, what is consistent with a recent report by Marks et al. (6). This is an observational study, and thus cannot rule out the presence of other potential confounders not controlled in the analysis, in particular the presence of helminth infection that can exacerbate or protect against allergic diseases depending on the burden of infection (26).

We believe that the most likely interpretation is that BCG only has an effect in reducing asthma with an atopic component (as is asthma with allergic rhinitis). The result from this study was similar to the study reported by Marks et al. (6), in which neonatal vaccination was associated with low prevalence of current asthma among

those aged 7–14 years and with family history of atopy (corresponding vaccine protection of 54%). Different mechanisms might be involved in the development of atopic asthma and of allergic rhinitis, with BCG modulating more effectively the one behind asthma. Asthma is a syndrome including different disorders characterized by airflow obstruction and wheezing-associated illnesses. Martinez et al. have classified clinical 'asthma' into three groups: early onset transient wheezers, persistent wheezers, and late-onset wheezers (27), with atopy associated with the second and third forms. Because ISAAC questionnaire depends on recall, it is likely that this study was better at detecting late-onset than the early-onset asthma. And why not protection against allergy and wheezing alone? It is possible that immunoglobulin E production and mast cell sensitization/activation after exposure to an allergen is not the only mechanism involved in the development of asthma and rhinitis – in other words they may share the immunopathogenesis to a certain extent only. This is consistent with recent findings in which deletion of a transcription factor, named T-bet, can be involved in the causation of asthma even with no need for allergen (28). T-bet is a factor necessary to make T_H1 lymphocytes produce interferon- γ . If this was the case, than one interpretation of the results would be that

neonatal BCG modulates a nonshared mechanism, which is only relevant for asthma.

Conclusion

In the population we surveyed, neonatal BCG scar was associated with a reduction in the risk of asthma only in individuals with a history suggestive of allergic rhinitis. Future prospective studies should be planned to investigate better the complexity of any protection given by BCG, by using objective measurements of atopy, rhinitis and asthma, taking environmental aspects into consideration.

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