

Early BCG vaccination and reduction in atopy in Guinea-Bissau

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Abstract

Background It has been proposed that certain viral and bacterial infections in early childhood may prevent allergic sensitization, by inducing Th1-type immune responses. This has led to speculation that mycobacterial vaccines might, through their Th1-stimulating properties, also protect against atopy.

Objective To investigate whether the prevalence of atopy is lower in children who have been vaccinated with BCG in infancy than in children who have not been vaccinated.

Methods We measured skin test reactivity to three allergens (*Dermatophagoides pteronyssinus*, *D. farinae* and cockroach) in 400 children, aged 3–14 years, as part of a follow-up study to examine the immune sequelae of measles in an urban area of Bissau, the capital of Guinea-Bissau in west Africa. Information on childhood vaccinations, including BCG in infancy, was available from child records. Of these children, 271 had been vaccinated with BCG (according to records) and 53 had not been vaccinated (no record and no BCG scar). Atopy was defined in two ways, according to the presence of any allergen reaction ≥ 2 mm and any reaction ≥ 3 mm.

Results Of the children who had received BCG vaccine, 57 (21%) were atopic (any reaction ≥ 2 mm), compared with 21 (40%) of the unvaccinated children [odds ratio, after controlling for potential confounding factors, 0.19 (95% CI 0.06–0.59)]. When atopy was defined using the 3-mm criterion, the reduction in atopy associated with BCG was greater the earlier the age at vaccination, and the largest reduction was seen in children vaccinated in the first week of life.

Conclusion BCG vaccination given early in infancy may prevent the development of atopy in African children.

Keywords: Africa, atopy, BCG vaccination in infancy, children

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Introduction

Epidemiological studies have provided indirect evidence [1,2] for the hypothesis that infections in early childhood

may prevent atopy and atopic disease. More direct evidence has come from recent studies of specific infections, including measles [3] and hepatitis A [4]. It has been hypothesized that viral and intracellular bacterial infections could inhibit the T helper 2 (Th2)-type immune response associated with atopic reactions by stimulating a Th1 response involved in delayed-type hypersensitivity reactions [5].

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These observations are important as they suggest that the secular decline in childhood infection in developed countries may have contributed to the rise in prevalence of atopic disease in recent decades [6]. However, they also raise questions about the possible impact of vaccinations in early childhood [3,7]. On the one hand, vaccinations might promote atopy, either through direct immune effects or by preventing the protective effects of natural infection. On the other hand, they might protect against atopy by inducing similar Th1 cytokine responses.

Since BCG triggers a marked Th1 response in adults and in animal models [8–11], we examined whether vaccination with BCG during infancy is protective against subsequent atopy in an urban community in Guinea-Bissau.

Methods

Study population

The study was carried out in 1994 in three districts in the capital of Guinea-Bissau. These have been followed with demographic surveillance since 1978 (Bandim 1) and 1984 (Bandim 2 and Belem) [12]. The major objective was to examine the impact of measles and intensity of exposure on cellular immunity but at the same time we also measured atopy. Details of the selection of the study sample and methods of investigation have been described previously [13]. Briefly, we included children aged 3–14 years who had measles documented in the 1991 epidemic in Bissau, and were still living there in 1994, as well as vaccinated controls. Having identified a secondary measles case, a primary case and a control of a similar age were selected from the same house as the secondary case or from neighbouring houses. All those invited to participate took part. Children were seen at home between May and September. Weight, height, mid-upper arm circumference and head circumference were measured and the children were examined for splenomegaly and the presence of BCG-like scars. A subgroup of 114 children, who were assessed during the rainy season, had a finger-prick blood test for malaria parasitaemia. A questionnaire was administered to the mother or guardian, which asked about risk factors for atopy and background socio-demographic information [13]. Birth weight data were available from records in a subgroup of 151 children who had been born in hospital.

Information on vaccinations was obtained from the inspection of health cards during the home visit and from the project population register, which collects information on growth, infections and vaccinations every 3 months from children under 3 years of age living in the study area. Many of the older children who had moved to the study area later in childhood had no vaccination record. We classified BCG vaccination status as follows: documented vaccination

irrespective of whether BCG-like scar present; BCG-like scar present but no documented vaccination; no scar and no documented vaccination. Since the vaccination information is likely to be much better for the children followed by the routine registration system, we carried out a separate analysis for the group of children born in the study area. Throughout the period when the study children were infants, the official policy had been to vaccinate with BCG from birth, with three doses of diphtheria, tetanus and pertussis (DTP) and polio vaccines between 1 and 4 months of age (given together), and with measles vaccine at 9 months of age. However, BCG vaccination only became common in Bissau from 1982 onwards as the national vaccination programme became more organized.

Skin-prick testing was done with three allergens, *Dermatophagoides pteronyssinus*, *D. farinae* and Cockroach mix (Dome/Hollister/Stier, Spokane, Washington, USA). In a previous study of older individuals in Bissau we found that individuals did not react to other aeroallergens including cat, dog, grass mix and aspergillus [3]. The allergens were applied with a negative saline control and a positive histamine control (10 mg/mL) to the volar aspect of the right forearm using separate 25 gauge needles. The perpendicular diameters of each weal were recorded after 10 min. One physician (CBH) carried out all skin tests and was blind to information on BCG. Atopy was defined in two ways: firstly, by the presence of any allergen reaction ≥ 2 mm (average diameter of weal minus reaction to negative control); secondly, by the presence of any reaction ≥ 3 mm.

Statistical analyses

Chi-squared test for difference in proportions was used to examine the prevalence of background factors according to BCG status. Logistic regression was used to examine associations with atopy, controlling for potential confounders. All analyses were performed using the statistical package SAS, version 6.12, SAS Institute Inc, Carry, NC, USA.

Results

Of the 405 children enrolled in the study of cellular immunity, skin-prick testing was not performed on five children. Of the remaining 400 children, 271 (68%) had documentation of BCG vaccination given at a median age of 12 days (range, 0–3072 days). Among the remaining 129 children, 76 had a BCG-like scar but no documentation and 53 had no scar and no documentation. The distribution of selected background factors according to BCG vaccination status is shown in Table 1.

Of the 400 children, 105 (26.3%) had one or more reactions ≥ 2 mm and 37 (9.3%) had one or more reactions ≥ 3 mm. The prevalence of atopy (any reaction ≥ 2 mm)

Table 1. Prevalence (%) of background factors according to BCG vaccination status in Bissau children

Background factor	BCG vaccination status			P-value*
	Documented % (n)	Scar only % (n)	Not documented/ no scar % (n)	
Males	51 (139/271)	49 (37/76)	49 (26/53)	0.899
Current age (years)				0.001
3–4	29 (79/271)	5 (4/76)	8 (4/53)	
5–6	34 (92/271)	14 (11/76)	21 (11/53)	
7–8	24 (64/271)	39 (30/76)	28 (15/53)	
9–14	13 (36/271)	41 (31/76)	43 (25/53)	
District (Bandim 1)	29 (78/271)	14 (11/76)	25 (13/53)	0.040
Testing dry season	27 (74/271)	20 (15/76)	23 (12/53)	0.364
Born in hospital	63 (171/270)	64 (47/73)	47 (24/51)	0.077
Born outside Bissau	10 (26/271)	15 (11/73)	33 (17/51)	0.001
Mother's schooling (years)				0.701
None	43 (116/271)	46 (35/76)	47 (25/53)	
1–3	14 (38/271)	17 (13/76)	9 (5/53)	
4+	43 (117/271)	37 (28/76)	43 (23/53)	
0–2 older siblings	61 (165/271)	61 (46/76)	55 (29/53)	0.700
Measles infection	64 (173/271)	79 (60/76)	92 (49/53)	0.001
Measles vaccination	87 (237/271)	57 (43/76)	34 (18/53)	0.001
DTP/polio vaccinations	96 (261/271)	12 (9/76)	11 (6/53)	0.001

* P-values from chi-square test.

according to BCG vaccination and selected background factors is shown in Table 2. Atopy was more common in older children and less common in children tested in the rainy season. Atopy was not significantly associated with height, weight, arm circumference or head circumference, when age and sex were taken into consideration, nor with malaria parasitaemia or splenomegaly. Atopy was associated with lower birth weight ($P = 0.026$) in the subset of 151 individuals with information.

Of 271 children with documented BCG vaccination, 57 (21%) were atopic compared with 21 (40%) of 53 children who had not been vaccinated (no documentation and no scar) (univariate OR 0.41 [95% CI: 0.22–0.76]) (Table 2). Amongst children with a documented BCG vaccination, the prevalence of atopy did not differ according to scar status. The effect of BCG on atopy was similar whether vaccination had been carried out in the dry or the rainy season. Children who had received BCG were also less likely to have any reaction ≥ 3 mm (OR 0.42 [0.18–1.08]).

The effect of BCG on atopy (any reaction ≥ 2 mm) became stronger after controlling for potential confounding factors in 394 children with complete information (OR 0.19 [0.06–0.59]) (Table 2). Although DTP/polio vaccination was associated with a nonsignificant reduction in atopy univariately, in the multivariate analysis it was associated

with an increase in atopy (OR 2.88 [1.01–9.02]). The effect estimate for BCG on atopy did not change if we controlled for age as a continuous variable, nor was it diminished by controlling additionally for birth weight in the subgroup with that information. As we obtained a similar effect estimate for BCG when controlling just for age, sex, DTP/polio vaccination and season of testing [OR 0.17 (0.05–0.51)], only these variables were included in subsequent multivariate analyses. When we examined the effects of BCG and DTP/polio vaccinations on the prevalence of any reaction ≥ 3 mm, the adjusted odds ratios were, respectively, 0.26 (0.06–1.17) and 2.00 (0.52–7.13).

The association between BCG and atopy became stronger when we restricted the analysis to 163 children who were born in the study area and followed regularly during the first three years of life, and therefore had the most reliable information on vaccination status (univariate OR 0.23 [0.07–0.71] and 0.13 [0.04–0.48], for any reaction ≥ 2 mm and ≥ 3 mm, respectively). These results were unchanged in multivariate analyses.

Table 3 shows the effect of BCG on atopy according to age at vaccination. There was some evidence that the reduction in atopy was greater the earlier the age at BCG vaccination (trend $P = 0.25$ for any reaction ≥ 2 mm; trend $P = 0.032$ for any reaction ≥ 3 mm), with the greatest

Table 2. Atopy* according to BCG vaccination and background factors in Bissau children

Background factor	Atopy	OR† (95% CI)	Adjusted OR‡ (95% CI)
<i>Sex</i>			
Male	25 (50/202)	1.0	1.0
Female	28 (55/198)	1.17 (0.75 to 1.83)	1.10 (0.68 to 1.75)
<i>Current age (years)</i>			
3–4	21 (18/87)	1.0	1.0
5–6	23 (26/114)	1.13 (0.58 to 2.26)	1.17 (0.58 to 2.41)
7–8	28 (30/109)	1.46 (0.75 to 2.88)	1.23 (0.60 to 2.56)
9–14	34 (31/90)	2.01 (1.03 to 4.02)	1.34 (0.62 to 2.93)
<i>District</i>			
Bandim 1	25 (26/102)	1.0	1.0
Bandim 2/Belem	27 (79/298)	1.05 (0.64 to 1.79)	0.91 (0.49 to 1.72)
<i>Season of testing</i>			
Dry (May)	34 (34/101)	1.0	1.0
Rainy (June–Sept)	24 (71/299)	0.61 (0.38 to 1.01)	0.57 (0.32 to 1.01)
<i>Place of birth</i>			
Home	27 (41/152)	1.0	1.0
Hospital	26 (62/242)	0.93 (0.59 to 1.48)	0.95 (0.58 to 1.58)
In Bissau	25 (86/341)	1.0	1.0
Outside Bissau	31 (17/54)	1.36 (0.72 to 2.51)	1.26 (0.63 to 2.46)
<i>Mother's schooling (years)</i>			
None	26 (45/176)	1.0	1.0
1–3	23 (13/56)	0.88 (0.42 to 1.75)	0.89 (0.41 to 1.83)
4+	28 (47/168)	1.13 (0.70 to 1.83)	1.19 (0.70 to 2.05)
<i>Older siblings</i>			
0–2	29 (69/240)	1.0	1.0
3–9	23 (36/160)	0.72 (0.45 to 1.14)	0.78 (0.47 to 1.28)
<i>Measles infection</i>			
No	21 (25/118)	1.0	1.0
Yes	28 (80/282)	1.47 (0.89 to 2.49)	1.21 (0.68 to 2.17)
<i>Measles vaccination</i>			
No	30 (31/102)	1.0	1.0
Yes	25 (74/298)	0.76 (0.46 to 1.25)	1.01 (0.54 to 1.93)
<i>DTP/polio vaccinations</i>			
No	32 (40/124)	1.0	1.0
Yes	24 (65/276)	0.65 (0.41 to 1.04)	2.88 (1.01 to 9.02)
<i>BCG vaccination</i>			
Not documented/no scar	40 (21/53)	1.0	1.0
Scar only	36 (27/76)	0.84 (0.41 to 1.74)	0.94 (0.42 to 2.08)
Documented	21 (57/271)	0.41 (0.22 to 0.76)	0.19 (0.06 to 0.59)

*Atopy defined as reaction (≥ 2 mm) to any of the allergens *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and Cockroach mix; †univariate odds ratio; ‡controlling for all other variables in the Table in 394 children with complete information.

reduction in children who had been vaccinated in the first week of life.

Discussion

This study has shown a strong association between BCG vaccination and atopy in a population-based study of children in Guinea-Bissau. Children who had been vaccinated with

BCG in early infancy were much less likely to be atopic on skin testing at 3–14 years of age than children who had not been vaccinated.

Previous studies of allergen skin testing in children have used either a 2-mm or a 3-mm weal to define atopy. The 2-mm criterion is likely to be more sensitive when studying children as young as 3 years, in whom skin test reactivity is lower than in older children [14]. Atopy defined in this way

Table 3. Atopy* according to age at BCG vaccination in Bissau children

Vaccination status (age)	Any reaction ≥ 2 mm		Any reaction ≥ 3 mm	
	% (n)	OR† (95% CI)	% (n)	OR† (95% CI)
Documented				
0–7 days	16 (16/97)	0.13 (0.04 to 0.41)	3 (3/97)	0.11 (0.02 to 0.63)
8–31 days	21 (21/99)	0.18 (0.05 to 0.58)	7 (7/99)	0.28 (0.06 to 1.39)
32–182 days	27 (13/48)	0.22 (0.06 to 0.76)	10 (5/48)	0.34 (0.06 to 1.86)
183+ days	26 (7/27)	0.20 (0.05 to 0.75)	15 (4/27)	0.52 (0.09 to 3.06)
Scar only	36 (27/76)	0.84 (0.40 to 1.77)	13 (10/76)	0.91 (0.32 to 2.62)
Not documented/no scar	40 (21/53)	1.0	15 (8/53)	1.0

*Atopy defined as reaction (≥ 2 mm or 3 mm) to any of the three allergens *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and Cockroach mix; †controlling for age, sex, season of testing and DTP/polio vaccinations.

is associated with an increased risk of atopic disease. However, as risk increases further with larger weals [15], we also examined the effect of BCG on atopy defined using a 3-mm criterion. A similar effect was seen using both definitions.

We think it unlikely that the findings have arisen through biased misclassification of BCG history as such misclassification is likely to be random with respect to atopy and this would tend to lead to an underestimation of effect. In keeping with this, we confirmed a stronger effect of BCG in the subgroup of children who were born in the study area and who had the most accurate and complete documentation of BCG vaccination. We classified BCG vaccination status primarily using information from vaccination records because this is likely to be more reliable than using the presence of BCG scar alone. Not all vaccinated children develop a scar, particularly if vaccinated early in life [16]. A study in Africa found that less than 60% of children who had been vaccinated in infancy had a detectable scar 3 years later. Furthermore, that study noted that reading of BCG scars may be difficult [17]. Thus some true scars may be missed. We found that amongst children with documented vaccination the prevalence of atopy was similar in those with and without a scar. There are two possible reasons why children with an apparent scar but no documented vaccination had a nonsignificant reduction in atopy when compared with unvaccinated children. Firstly, a proportion of the scars may have been 'false-positives', unrelated to BCG vaccination. Such misclassification could lead to an underestimation of effect. Secondly, some of these children may have been vaccinated after infancy when BCG vaccination may have less impact on atopy. In support of this, these children were older (mean age 8.2 years) than children with documented vaccination (mean age 6.0 years). Hence they were born before, and likely to have been vaccinated during, the accelerated expanded vaccination

campaigns in 1986–1987, when older age groups were also vaccinated and documentation of vaccination was often poor. They were also more likely to have been born outside the study area. In view of these differences and the unreliability of scar assessment we believe that it was appropriate to treat the 'scar only' children as a separate group in the analyses.

We found that the prevalence of atopy was lower in the rainy season, in younger children and in children who were of lower birth weight. A similar association between birth weight and atopy has been found in 2-year-old children [18] but not in adults [19] in Britain. However, the strong effect of BCG on atopy remained after controlling for these factors and could not be explained by other measured potential confounding factors. Whilst we cannot exclude the possibility that our findings are explained by unmeasured confounders, the magnitude of the BCG effect makes this less likely. Helminth infestation is common in African children and it has been proposed that this might suppress the expression of atopy by inducing high levels of total IgE that block mast cell receptors for allergen-specific IgE [20]. However, if parasitic infestation were to explain the association between BCG and atopy, then levels of helminths would have to be higher in children who had been vaccinated in infancy than in those who had not been vaccinated. We did not measure total IgE or worm burdens in our study but we think that such confounding is unlikely as we would expect worm burdens to be higher in unvaccinated children who are more likely to come from less sanitary homes and poorer families. As atopic children tend to come from more hygienic and affluent backgrounds, controlling for parasitic infestation and other measures of hygiene and socio-economic status would be expected to strengthen, not weaken, the association between BCG and atopy.

Recently, a Swedish study reported no effect of BCG given before 6 months of age on atopy or atopic disease

among children of atopic parents [21]. However, BCG may only prevent the development of atopy in the absence of a strong genetic influence [22]. A subsequent Swedish study of unselected children, using parental recall of BCG vaccination, found no reduction in atopic disease associated with BCG in infancy amongst children born in Sweden. However, amongst immigrant children, many born in Asia and South America, BCG was associated with a lower prevalence of atopic disease [23]. One interpretation of our findings and those from the Swedish studies is that BCG prevents atopy only in certain ethnic groups. Recent evidence suggests that genes which play a part in the expression of atopy may be influenced by ethnicity [24]. Alternatively, the effect of BCG may be modified by environmental factors that differ between the west and developing countries. Helminth infestation is uncommon in infancy in Guinea-Bissau [25] but the high burden of viral and bacterial infections in early life in Africa might enhance the Th1-inducing activity of BCG.

We found some evidence to suggest that the effect of BCG on atopy may be greater the earlier the age at vaccination, which would support a causal interpretation. The mechanism underlying the protective effect may involve activation of a Th1-type immune response during a critical period. It was recently reported that BCG suppresses allergen-induced airway eosinophilia and hyper-reactivity in mice by stimulating a Th1-type response [10,11]. In our study, the greatest reduction in atopy was observed in children who had been vaccinated during the first week of life. This observation may be surprising as newborns are considered to be Th2-biased and poorly able to develop Th1 responses [26]. However, we recently observed that BCG vaccination at birth triggers a Th1 response of similar magnitude to that seen with vaccination at 2 months of age [27], supporting the concept that newborns can develop Th1-type immune responses [28–30]. In order to influence the function of allergen-specific T lymphocytes, the Th1 activation induced by BCG would have to take place before, or at the time of first exposure and sensitization to allergens. The lowest prevalence of atopy was in children who were vaccinated in the first week of life and this may be because exposure and first sensitization to dust mite and cockroach allergens is most likely to occur at this time. Alternatively, the sensitivity of neonatal T-lymphocytes to Th1-activating agents may be different to that of lymphocytes from older children.

The majority of children vaccinated with BCG had also received DTP/polio vaccines. Consequently, in the univariate analysis DTP/polio vaccination was also associated with a reduction in the prevalence of atopy. On controlling for BCG vaccination, however, DTP/polio vaccination was associated with a higher prevalence of atopy, although the association was not significant when we used a 3-mm

definition. Given that DTP and polio vaccines are nearly always administered together it is not possible to ascribe the effect to one or other vaccine. Interest has focused on a possible link between pertussis vaccine and atopy because it boosts IgE production in animal models [31] and previous observational studies have found an increase in atopy and atopic disease in relation to whooping cough [32] and DTP vaccination [7,33].

In a previous study of a cohort of young adults aged 14–21 years, from the same community, measles infection was associated with a lower prevalence of atopy [3]. In contrast, we did not find a similar effect of clinical measles infection on atopy in the present study. However, the vaccinated 'control' groups were very different in the two studies. In the older cohort, none of whom had received BCG vaccine, controls had received measles vaccine at a mean age of 3 years. In the present study, controls, most of whom had been BCG vaccinated, had received measles vaccine under 1 year of age. Furthermore, many are likely to have had subclinical measles infection [34] through intensive exposure to measles cases in the same house during the 1991 epidemic. Hence, our study did not allow the same distinction between 'infected' and 'noninfected' children as was possible in the older cohort.

Holt has proposed that Th1-selective adjuvants might be used in the future as part of a vaccination strategy to prevent atopy and atopic disease [35]. Our findings suggest that BCG vaccination in infancy, and particularly in the first week of life, may have a potential role in such a strategy. We believe that a randomized trial to study the effects of BCG vaccination at different ages on atopy and atopic disease is warranted.

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Contributors

SOS, PA, and AJH planned the study of immune sequelae of measles infection. Field interviews and clinical examinations were conducted by CBH, AG, and SOS. PA and AM

planned the reanalysis of impact of age at BCG vaccination and PA conducted additional data collection. Statistical analysis was carried out by HJ and AWS. PA and SOS wrote the first draft of the present paper and all authors have contributed to the final version.

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