

Absence of association between delayed type hypersensitivity to tuberculin and atopy in children in The Gambia

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Summary

Background An inverse association between delayed type hypersensitivity to tuberculin and atopy has been observed in children, suggesting that exposure to mycobacteria may influence the immune response to allergens.

Objective To investigate the relationship between tuberculin responses and atopy in children living in three different environments in The Gambia.

Methods In this cross-sectional study a total of 507 school-aged children were recruited from rural, urban poor or urban affluent communities. They were assessed for skin responses to five common allergens and tuberculin, presence of bacille Calmette–Guérin (BCG) scar, presence of intestinal parasites, and total serum IgE. Atopy was defined as the presence of a skin prick test response $\geq 3 \times 3$ mm to at least one allergen.

Results The overall prevalence of atopy was 33% but there was a significant variation among the three study groups. The prevalence of atopy was 22% in urban poor, 36% in urban affluent, and 43% in rural children. Controlling for potential confounding factors, children in the rural community had a significantly higher odds ratio, 3.3 (95% confidence interval 1.8–6.0) of being atopic than children from the urban poor community. No association between atopy and tuberculin response or BCG scar was observed in any of the three groups. Serum IgE levels were higher among children of the urban poor group but were not associated with tuberculin response or BCG scar in any of the groups.

Conclusion Environmental factors have an important influence on the development of atopy in children in The Gambia but delayed type hypersensitivity to tuberculin is not a protective factor.

Keywords atopy, children, environment, The Gambia, tuberculin

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Introduction

The prevalence of atopic diseases has increased in many parts of the world during the last decades, especially in industrialized countries [1, 2]. Although genetic factors play an important role in the susceptibility to atopy, this rapid increase is probably related to changes in environmental factors [2, 3]. The hygiene hypothesis postulates that infections in early life influence the development of allergic diseases [2–4]. This hypothesis is supported by studies showing an inverse association between atopic disorders and exposure to infectious pathogens in early life [5–9]. In school children in Japan, an inverse association between delayed type hypersensitivity to tuberculin and atopy was observed, suggesting that mycobacterial exposure may influence the immune response to allergens [7]. Mycobacteria are among the most potent inducers of T helper type 1 (Th1)

responses and could inhibit the development of pathogenic Th2 responses to allergens. Supporting this hypothesis, early bacille Calmette–Guérin (BCG) vaccination was associated with a reduced risk of atopy in childhood in Guinea-Bissau [10], a similar effect but limited to first 2 years of life in Germany [11], but had no effect in other studies [12, 13].

In some African countries, although exposure to infectious pathogens is very high in early life, the prevalence of atopy in children is also relatively high [9, 14, 15]. Interestingly, this high prevalence of atopy is associated with a relatively low prevalence of asthma. The possible association between atopy and mycobacterial infections in African children remains poorly defined. In addition to BCG, children in Africa have high exposure to *Mycobacterium tuberculosis* and environmental mycobacteria [16]. This study was undertaken to evaluate the possible association between atopy and delayed hypersensitivity to tuberculin in Gambian children living in rural, urban poor, or urban affluent communities. Given that atopy is usually associated with increased circulating allergen-specific IgE, we also studied the relationship between IgE, skin prick test (SPT) response to allergens and mycobacterial infections in the study communities.

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Materials and methods

Study population

This cross-sectional study was approved by The Gambia Government/Medical Research Council (MRC) Ethics Committee. Children aged between 8 and 12 years representing three different social strata and exposed to different environments were enrolled into this study during the rainy season (July to November 2000), after obtaining parental consent. Children attending Marina International School in Fajara represented a high income urban affluent (UA) group, while those from Bakau Primary School, which is located only 500 meters away from the former, represented an urban poor (UP) population. These schools are in the coastal area of The Gambia. Children dwelling in a rural area (R) were recruited from 12 villages and hamlets in the catchment area of Sinchu Njabo Primary School (only 17% of children in this area attend school) located on the north bank of the River Gambia and 170 km from the coast. Children from the urban area were randomly selected by choosing the odd numbers from their respective class lists, while in the rural area they were sampled from alternate compounds according to the MRC demographic surveillance site database [17]. The response rate was above 90% in all three communities studied and did not vary significantly among the three communities. Also, the outcome parameters did not depend on school attendance in the rural community. Subjects were eligible for the study if they had lived at least 2 years in the area. Parents or caretakers were asked about basic demographic data of the child, vaccination history and crowding index of household. Mobile clinics were organized for the rural villages, while in the urban area the children were seen at their schools. At these visits a general medical examination was performed, presence of BCG scar was recorded, and weight and height measured.

Skin response to allergens and tuberculin

Skin responses to five common allergens including *Dermatophagoides pteronyssinus*, cockroach mix (American and German), mold mix (including *Aspergillus*, *Alternaria*, *Penicillium*), grass mix and epidermal mix (Bayer Allergy, Spokane, WA, USA), histamine, and glycerol controls were measured on the volar surface of the left forearm. Vertical and horizontal diameters of positive reactions were measured after 15 min. A positive response was defined as a mean diameter above or equal to 3 mm. Subjects with a positive reaction to at least one allergen were defined as atopic. We determined the skin response to tuberculin (PPD, RT23, Statens Serum Institute, Copenhagen, Denmark) and *Candida albicans* (Evans Medical Ltd, London, UK) administered intradermally at two separate sites on the volar surface of the right forearm [18] by measuring the vertical and horizontal diameters of reactions after 48–72 h.

Laboratory measurements

Stool samples were concentrated within a few hours of collection and examined by light microscopy after saline mounting for the ova of geohelminths including *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm, and *Strongyloides stercoralis*.

Serum was separated and stored in liquid nitrogen within 1 h of collection and thereafter stored at -70°C until measurement. Total IgE concentration was measured using the CAP System

IgE FEIA method (Pharmacia, Uppsala, Sweden) following the manufacturer's instructions.

Statistical analysis

Logistic regression was used to calculate odds ratios (OR) for the prevalence of atopy. The distribution of serum IgE was skewed but was normalized by logarithmic transformation before analysis. Analysis of variance was used to compare the group means of the transformed IgE variable. Bootstrapping methods were used to approximate 95% confidence intervals (CI) for mean differences on the original scale. All analyses were performed using GenStat version 5 (Oxford, UK).

Results

Children living in the rural area had a higher prevalence of atopy

A total of 507 children participated in the study, with 193 classified as R, 196 classified as UP, and 118 classified as UA. Overall, 33% of the children were atopic and there was a significant difference ($P < 0.001$) among the proportions of atopic subjects in each group. Highest prevalence of atopy was detected among children of the R group (43%), whereas the lowest prevalence was observed among UP children (22%). UA children had intermediate prevalence of atopy (36%). Table 1 shows the prevalence of atopy for each measured variable, where IgE has been grouped into high or low according to whether it is above or below the median IgE value, respectively. The first column of Table 1 shows the variables that were recorded for each child. A univariate logistic regression analysis was used to assess the influence of the measured parameters on the incidence of atopy. The incidence of atopy was consistently higher in those subjects with high IgE levels. The OR of atopy prevalence for those with high levels of IgE compared to those with low levels, in the rural, urban poor and urban affluent groups, were OR 2.3 (95% CI 1.3–4.1), OR 2.5 (95% CI 1.2–5.1) and OR 4.3 (95% CI 1.9–9.8), respectively. Within the R group no other parameters were significant. For the UP group, age was a significant effect, with 11- to 12-year-old having a significantly higher probability of atopy than 8-year-old (OR 3.4, 95% CI 1.1–11.0). Subjects within the UA group with doctor-diagnosed asthma had a significantly higher chance of being atopic, compared to those subjects without a diagnoses of asthma (OR 4.3, 95% CI 1.9–9.8). There was no significant association in the groups between atopy and skin response to tuberculin. The inference was the same if the tuberculin responses were divided into two groups, defined as below and above the median response (data not shown). The overall response to *Candida* was 91%, with no significant variation across the groups (data not shown). Presence of BCG scar, history of BCG, or diphtheria-pertussis-tetanus (DPT) or oral polio (OPV) vaccination had no significant effect on atopy in any of the groups.

After adjusting for all possible confounders in Table 1 (IgE and doctor-diagnosed asthma) and comparing groups to UP children, the OR for developing atopy were OR 3.3 (95% CI 1.8–6.0) in the R group and OR 2.6 (95% CI 0.7–10.2) in the UA children. The most common sensitizing allergen was *D. pteronyssinus*, followed by cockroach mix and grass mix.

Table 1. Prevalence of atopy in the presence of potential risk factors among the three study groups

Effect	Urban affluent	Urban poor	Rural
Height (cm, means: yes/no)	141.6/141.7	136.7/134.1	132.8/133.2
Weight (kg, means: yes/no)	32.9/33.3	29.5/27.2	25.9/25.9
IgE low	17% (12/69)	14% (14/98)	33% (32/97)
IgE high	53% (31/59)*	30% (29/98)*	53% (51/96)*
Age 8	50% (12/24)	15% (5/34)	42% (14/33)
Age 9	21% (5/24)	20% (10/50)	40% (16/40)
Age 10	38% (11/29)	19% (9/47)	44% (18/41)
Age 11	37% (11/30)	37% (13/35)	37% (17/46)
Age 12	36% (4/11)	20% (6/30)	55% (18/33)*
Male	40% (24/60)	22% (20/91)	49% (47/95)
Female	33% (19/58)	22% (23/105)	37% (36/98)
Other	44% (7/16)	26% (34/129)	37% (22/60)
Mandinka	27% (14/51)	12% (1/8)	44% (37/85)
Wolof	27% (3/11)	15% (5/34)	43% (13/30)
Fula	47% (19/40)	12% (3/25)	61% (11/18)
Wheezing	53% (9/17)	32% (6/19)	36% (4/11)
No wheezing	34% (34/100)	21% (36/173)	43% (77/179)
Doctor-diagnosed asthma	65% (13/20)*	27% (4/15)	50% (1/2)
No doctor-diagnosed asthma	31% (30/97)	21% (36/174)	43% (81/189)
History of asthma	39% (15/38)	18% (6/34)	43% (3/7)
No history of asthma	35% (26/75)	21% (32/150)	44% (76/174)
Doctor-diagnosed eczema	45% (5/11)	40% (2/5)	20% (1/5)
No doctor-diagnosed eczema	36% (37/103)	21% (40/189)	43% (80/185)
BCG scar	41% (37/90)	24% (36/150)	38% (46/120)
No scar	23% (6/26)	17% (7/41)	50% (35/69)
Mantoux < 3 mm	36% (34/94)	23% (31/132)	42% (52/125)
3 mm ≤ Mantoux < 6 mm	43% (3/7)	28% (5/18)	37% (3/8)
6 mm ≤ Mantoux < 10 mm	20% (2/10)	16% (3/19)	24% (4/17)
Mantoux ≥ 10 mm	57% (4/7)	15% (4/27)	56% (24/43)
Worms	75% (3/4)	17% (13/76)	43% (19/44)
No worms	35% (40/114)	25% (30/120)	43% (64/149)
Fewer than two to a room	32% (25/77)	14% (6/43)	32% (13/40)
Between two and four to a room	45% (17/38)	26% (30/114)	45% (42/93)
More than four to a room	33% (1/30)	18% (7/39)	47% (28/60)
Islamic	0% (0/1)	25% (20/81)	45% (68/151)
Non-formal	—	20% (14/71)	35% (12/34)
Primary	50% (1/2)	17% (4/23)	0% (0/4)
Secondary	40% (21/52)	25% (5/20)	50% (1/2)
Technical	41% (11/27)	—	—
University	29% (10/35)	0% (0/1)	—
Rugs	36% (34/94)	25% (1/4)	—
No rugs	37% (9/24)	22% (42/191)	42% (81/191)
Measles infection	21% (6/29)	22% (8/36)	44% (4/9)
No infection	40% (34/84)	21% (30/146)	43% (78/183)
Vaccination	38% (43/114)	21% (36/172)	42% (74/175)
No vaccination	0% (0/2)	33% (1/3)	50% (2/4)

*Significant risk factor for atopy on univariate analysis. Missing values are as a result of incomplete records.

Main risk for atopy is high IgE

Table 2 shows results of crude and adjusted OR for the determinants of atopy in each of the three study groups. The adjusted OR were calculated by retaining only those effects that were significant at the 5% level, after allowing for all other variables

in the model. The risk of atopy was significantly higher in all three groups of children within the high IgE categories. Adjusting for IgE concentrations also increased the risk of atopy among Fula children of the rural group, but age no longer had a significant effect on atopy in the UP children. After

Table 2. Crude and adjusted* odds ratios for significant determinants of atopy in the three study groups

Study group	Effect	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Urban affluent			
IgE	Low	1.0	1.0
	High	4.3 (1.9–9.8)	3.8 (1.6–9.0)
Asthma	No asthma	1.0	1.0
	Diagnosed	4.15 (1.36–12.95)	4.3 (1.9–9.8)
Urban poor			
IgE	Low	1.0	1.0
	High	2.5 (1.2–5.1)	2.6 (1.4–4.7)
Rural			
IgE	Low	1.0	1.0
	High	2.3 (1.3–4.1)	2.6 (1.4–4.7)
Ethnicity	Other	1.0	1.0
	Fula	2.7 (0.9–8.0)	3.5 (1.2–10.8)

*Adjusted for all variables significant at 5% level.

adjusting for IgE concentrations, the probability of being atopic was higher among UA children in whom a doctor had diagnosed asthma previously compared to their non-asthmatic counterparts (OR 4.3, 95% CI 1.9–9.8). The absence of association with asthma among UP children could be related to very low numbers as a result of limited access to appropriate diagnosis.

Serum IgE concentrations varied with study population

The IgE concentrations were highly skewed and Table 3 shows the median (lower, upper quartile) total serum IgE concentrations according to risk factors in the three study groups. As expected, atopic children had higher median IgE concentrations in the three study groups. Children from the R and UP groups with worms had lower median IgE concentration compared to those without worms; in contrast, children in the UA group with worms had a higher median IgE concentration but none of these differences was statistically significant.

T-tests based on log_e (IgE concentrations) showed that the mean IgE concentrations of atopic children were significantly (at 5% level adjusted for multiplicity) higher than those of non-atopic children within all three groups. The mean IgE differences between the atopic and non-atopic subjects and their bootstrapped 95% CI were 532 IU/mL (95% CI 290–796 IU/mL), 382 IU/mL (95% CI 9–793 IU/mL) and 660 IU/mL (95% CI 180–1216 IU/mL) for UA, UP and R groups, respectively.

Surprisingly there was no significant association between IgE concentration and tuberculin response in any of the three groups. No significant effect was found for the other variables in any of the groups.

Multiple linear regression based on group, showed a significant difference in mean IgE concentrations between the three groups ($P < 0.001$). After adjusting for multiplicity, UA children had significantly lower mean IgE values than UP children ($P < 0.001$) and R children ($P < 0.001$). The mean difference between the UP and UA groups was 510 IU/mL, with a bootstrapped 95% CI of 174–927 IU/mL; and for the UA and R groups the mean difference was 341 IU/mL, with a bootstrapped 95% CI of 153–523 IU/mL. No significant difference

was found between the mean IgE levels of UP and R children ($P = 0.3$).

Discussion

This study shows that the prevalence of atopy in children in The Gambia varies according to the community in which they are living but is not associated with delayed hypersensitivity to tuberculin. Similarly, total IgE concentration varied significantly according to the community. Atopy was associated with increased total serum IgE concentration in children in every community. However, the communities with higher IgE titers did not necessarily have a higher prevalence of atopy, implying that factors other than allergens in the environment contribute to IgE production.

Children of rural communities had a higher prevalence of atopy than children of urban communities. In rural communities, Fula children had a higher risk of being atopic than children of the other ethnic groups. However, the difference in prevalence of atopy between R and UP children was still significant after adjusting for ethnicity. Although the higher prevalence of atopy in the R community as compared to UP contrasts with what has been described in Western countries and earlier rural–urban studies in Africa [19, 20], it is in agreement with studies done in Ethiopia [9, 15]. Given that rural communities in The Gambia have been reported by Godfrey et al. to have a higher load of house dust mite [21], which is the most frequent allergen in our study, the higher prevalence of atopy observed could at least be partly related to a more intense exposure. However, an opposite observation was made in rural communities in Ethiopia where prevalence of atopy was higher but the load of dust mite was lower than in the urban environment [15]. Together, these data indicate that the development of atopy following exposure to allergens depends on the interaction of a number of factors. The differences in prevalence of atopy and IgE titre between the UP and UA groups attending schools separated by only 500 meters but living in different environments and social status suggest that environmental factors could be contributory to the differences in the prevalence of childhood atopy. Moreover, at the time of Godfrey's report the prevalence of atopy was very low despite the high load of allergen in the rural communities. We speculate that the cause of an increase in the prevalence of atopy in the rural community compared to the past could be related to a decline with time of factors that might have limited the development of atopy. Scrivener et al. [9] have suggested that a higher burden of intestinal parasites is protective against atopy. Perhaps increasing levels of sanitation with time in the rural communities could have reduced the infectious disease burden, thereby lowering its protective effect over the development of atopy. Although there was no significant difference in the prevalence of intestinal parasites, other infectious pathogens like hepatitis A virus or *Schistosoma haematobium* noted to have similar effects were not investigated in our study.

Atopy develops when the interaction of an allergen with the immune system leads to stimulation of Th2 cytokines such as IL-4, IL-5, and IL-13, which promote the production of IgE, eosinophils, and mast cells. The balance between Th2 and Th1 cytokines is pivotal as they reciprocally inhibit each other [22]. However, we did not find any evidence that conditions that

Table 3. Median (lower and upper quartile) IgE for selected parameters in the three study groups

Effect	Urban affluent	Urban poor	Rural
Atopic	541 (193, 1197)*	1056 (338, 1748)*	716 (264, 1480)*
Non-atopic	108 (55, 359)	511 (222, 1116)	353 (179, 740)
Age 8	444 (105, 1580)	838 (327, 1354)	499 (153, 1046)
Age 9	200 (65, 502)	600 (333, 1394)	797 (227, 1479)
Age 10	163 (83, 566)	430 (208, 852)	526 (222, 1355)
Age 11	211 (54, 563)	550 (306, 1412)	325 (179, 696)
Age 12	146 (63, 362)	594 (260, 1203)	461 (172, 1054)
Male	228 (102, 758)	550 (243, 1215)	461 (184, 1206)
Female	151 (54, 479)	542 (305, 1206)	488 (211, 1069)
Other	203 (116, 520)	604 (256, 1206)	465 (184, 947)
Mandinka	135 (56, 451)	528 (195, 1002)	624 (181, 1378)
Wolof	285 (99, 1073)	546 (327, 1789)	336 (179, 1034)
Fula	291 (75, 891)	504 (278, 1144)	346 (261, 576)
Wheezing	218 (97, 811)	532 (379, 862)	328 (125, 560)
No wheezing	215 (65, 573)	550 (235, 1214)	495 (191, 1126)
Doctor-diagnosed asthma	452 (138, 853)	506 (377, 861)	1437 (114, 2760)
No doctor-diagnosed asthma	172 (66, 553)	546 (254, 1214)	480 (185, 1087)
History of asthma	191 (86, 541)	795 (412, 1800)	188 (163, 1339)
No history of asthma	189 (62, 661)	503 (221, 1114)	488 (199, 1102)
Doctor-diagnosed eczema	121 (58, 1009)	365 (195, 1601)	694 (51, 1072)
No doctor-diagnosed eczema	204 (72, 576)	547 (262, 1214)	480 (185, 1118)
BCG scar	248 (73, 746)	532 (260, 1214)	428 (185, 1106)
No scar	117 (58, 332)	628 (351, 1159)	531 (186, 1313)
Mantoux < 3 mm	168 (73, 561)	537 (262, 1427)	495 (181, 1118)
3 mm ≤ Mantoux < 6 mm	785 (345, 1160)	610 (221, 1190)	1220 (178, 2700)
6 mm ≤ Mantoux < 10 mm	145 (46, 541)	628 (279, 1150)	337 (128, 637)
Mantoux ≥ 10 mm	443 (138, 718)	618 (341, 1100)	576 (276, 1168)
Worms	557 (120, 1587)	544 (246, 1487)	379 (151, 1262)
No worms	211 (66, 580)	553 (262, 1197)	512 (208, 1044)
Fewer than two to a room	204 (63, 460)	420 (220, 930)	315 (181, 687)
Between two and four to a room	310 (108, 769)	633 (333, 1324)	425 (185, 1118)
More than four to a room	58 (35, 90)	535 (206, 1448)	701 (211, 1262)
Islamic	—	613 (309, 1283)	480 (187, 1126)
Non-formal	—	550 (277, 1364)	622 (1069, 223)
Primary	—	353 (214, 598)	128 (84, 192)
Secondary	330 (92, 758)	641 (295, 1213)	730 (162, 1297)
Technical	332 (91, 680)	—	—
University	73 (48, 295)	—	—
Rugs	197 (62, 852)	670 (145, 1168)	—
No rugs	225 (100, 533)	547 (165, 1211)	480 (184, 1097)
Measles infection	121 (61, 329)	651 (231, 1088)	337 (247, 1021)
No infection	294 (87, 723)	522 (260, 1215)	495 (184, 1107)
Vaccination	211 (71, 580)	539 (222, 1214)	499 (187, 1113)
No vaccination	610 (18, 1201)	506 (480, 967)	250 (282, 1198)

*Significantly different on univariate analysis.

induce strong Th1 responses like mycobacterial and measles infections or BCG vaccination were associated with a reduction in the skin test response to allergens, or IgE titer, which are indicators of atopy. Several factors could have accounted for this. This could be related to the ubiquitous exposure to mycobacteria in our environment that could have diluted its effect on atopy, and one would expect to demonstrate its impact in other settings with lower levels of exposure. It is also likely that the time of exposure to mycobacteria or BCG vaccine, which our

study did not address, might be critical in influencing the development of atopy [10]. Delayed type hypersensitivity to tuberculin may not be a reliable marker of mycobacterial infection in our study population. On the other hand, our data and those of others showing a progressive increase in type 1 diabetes, a Th1-related disease, in the face of increasing prevalence of atopy in developed countries [23, 24], suggest that the pathogenesis of atopy could be more complicated than only a shift in Th1/Th2 balance.

We conclude that, in contrast to observations made in school children in Japan, delayed hypersensitivity to tuberculin is not inversely associated with atopy in children in The Gambia [7]. The comparison of affluent and poor urban communities in Africa should help us in understanding the environmental factors involved in the increased prevalence of allergic disorders worldwide.

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