

Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10

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Summary

Background Most of the effort directed at understanding the role infections have in preventing allergy has focused on bacteria and viruses and their ability to divert the immune system towards T-helper-1 responses and away from proallergic T-helper-2 responses. However, helminth infections, highly prevalent in large parts of the developing world, where allergy is uncommon, stimulate strong T-helper-2 responses. We investigated the influence of chronic helminth infections on the prevalence of atopy and aimed to understand the relation at a detailed immunological level.

Methods 520 Gabonese schoolchildren were tested for skin reaction to house-dust mite and other allergens, for *Schistosoma haematobium* eggs in urine, and for microfilariae in blood samples. Total and mite-specific IgE antibodies were measured. A subsample selected on the basis of their skin test to house-dust mite received detailed immunological investigations.

Findings Children with urinary schistosomiasis had a lower prevalence of a positive skin reaction to house-dust mite than those free of this infection (odds ratio 0.32 [95% CI 0.16–0.63]). The degree of sensitisation to house-dust mite could not explain this difference in skin-prick positivity. Schistosome-antigen-specific concentrations of interleukin-10 were significantly higher in infected children, and higher specific concentrations of this anti-inflammatory cytokine were negatively associated with the outcome of skin-test reactivity to mite (0.53 [0.30–0.96]). No association between polyclonal IgE antibodies and skin-test results was found.

Interpretation The anti-inflammatory cytokine, interleukin-10, induced in chronic schistosomiasis, appears central to suppressing atopy in African children.

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Introduction

The prevalence of atopic disorders is generally low in developing countries but has increased alarmingly in the industrialised world in the past few decades.¹ The concurrent improvement in sanitation and reduction in childhood infections in developed countries had led to the speculation that infections in early childhood may reduce the risk of allergy, the so-called “hygiene hypothesis”.² This theory gains support from the findings that children frequently exposed to infections in families with an anthroposophic lifestyle have fewer allergies.³ More direct evidence comes from epidemiological studies that show an inverse association between a variety of bacterial (*Mycobacterium tuberculosis*, *Helicobacter pylori*), viral (measles, hepatitis A), or protozoan (*Toxoplasma gondii*) infections and atopy.^{4–6} The mechanism proposed for this effect is that such infections would shift the balance of immune responses towards T-helper-1 (Th1), thereby reducing the expression of T-helper-2 (Th2) cytokines generally associated with allergy.⁷ Indeed, Japanese children with delayed hypersensitivity to tuberculin, a Th-1 type response, were less likely to have atopy.⁴ However, one important category of infections, which do not fit into the “Th1 shift” concept, needs to be considered. Helminths stimulate potent IgE responses, reflecting a strong Th2 expansion. In helminth-infested hosts, the balance of helper T-cell responses is more skewed towards Th2, when compared with that of European controls.⁸ Paradoxically, in countries where helminth infections have high prevalences and the immunological balance is shifted towards Th2-type responses, allergic disorders are least prevalent.¹ Thus the hygiene hypothesis, which argues that the lower rate of allergies in developing countries is caused by the multiplicity of childhood infections that shift immune responses to Th1, needs to be modified to accommodate helminth infections that are highly prevalent in these countries and, in fact, shift the overall responses towards Th2.

The diminished prevalence of allergic diseases in helminth-infected populations, despite highly raised concentrations of IgE and Th2 cytokines, may be due to certain features of these infections such as raised polyclonal IgE, high concentrations of antigen-specific IgG4, or heightened capacity to produce interleukin-10, an anti-inflammatory cytokine.^{9–11}

This paper presents the results of an immuno-epidemiological study done in Lambaréné, Gabon, aimed at furthering our understanding of the relation between helminth infections and allergy.

Methods

Study population

Lambaréné, Gabon, is an area with little urbanisation, high vaccine coverage, and high prevalence of helminth infections. This study presents data from two populations: a large group of 520 schoolchildren aged 5–14 years, and a subsample of 132 of these children selected on the basis of their reactivity to house-dust mite. The children included in the larger survey were attending one of three

schools in the area of Lambaréné, whereas children included in the smaller substudy were all from one school. Written informed consent was obtained from the parents of all participating children. The study was approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital.

Study design

The children were skin-prick tested, screened for parasite infections, and donated blood for serological examination. Standard skin-prick tests, which have been used in several epidemiological studies in Africa before, were done on the volar side of the child's lower arm with separate 25-gauge needles.^{12,13} Aeroallergens tested were extracts of *Dermatophagoides pteronyssinus*, *Dactylis glomerata* grass pollen, and preparations of cat dander and dog dander (HAL Allergen Laboratories, Netherlands). Histamine chloride (10 g/L) was used as the positive control, and allergen diluent as the negative control. The longest diameter (D1) of a wheal and the diameter perpendicular to it (D2) were measured after 10 min. A skin-prick test was regarded as positive when half the sum of D1 and D2 was greater than 3 mm.¹² The same batches of extracts were used and the skin-prick tests were done throughout the study by the same researcher. The presence of microfilariae (*Loa loa* and *Mansonella perstans*) was ascertained in thick blood smears, and the presence of schistosome infection (*Schistosoma haematobium*) by the detection of eggs in 10 mL filtered urine. A random group of 66 children were screened for infections with intestinal helminths by detecting eggs in faeces prepared according to the Kato method. 74% of these children were positive for *Ascaris lumbricoides* or *Trichuris trichiura*, or both. Concentrations of total IgE were measured by ELISA with rabbit antihuman IgE antibodies (Dako, Denmark) and goat antihuman IgE-biotinylated antibodies (Vector, CA, USA) as capture and detection antibodies, respectively. As a reference the WHO standard of human serum IgE (NIBSC, UK) was used, results being expressed in IU/mL. Serum mite-specific IgE was measured by radioallergosorbent test (RAST), with Sepharose-coupled *D. pteronyssinus* allergens and radiolabelled sheep antibodies directed to human IgE. Results were expressed in IU/mL, with a dilution series of chimaeric monoclonal IgE antibody against the major house-dust-mite allergen Der p 2 and Sepharose-coupled mite extracts as a reference.¹⁴ The detection limit of the RAST was over 0.3 IU/mL where 1 IU is 2.4 ng IgE.

A subsample of 132 children was included in a more detailed immunological study to shed light on the interaction between schistosome infections and allergy. All individuals with a positive skin-prick test for mite were asked to participate and 30 positive children attending the public school of Lalala consented to donate over 10 mL venous blood for cellular immunological studies. 231 children who had negative skin-prick tests in the large sample were selected at random to participate in the substudy and of these 102 consented to give blood. The distributions of age and sex, as well as of mite-specific and total IgE antibodies, were similar between positive children included in the subsample and those who were not. This also holds for the children with a negative skin-prick test. Questionnaires were used to collect data on demographic and lifestyle variables and on history of asthma and rhinitis. A local health worker asked the questions in the indigenous language. Neither hospital admission due to breathing problems nor any cases of family asthma were reported. No symptoms that could be classified as allergic rhinitis were documented. The

	Negative test (n=462)	Positive test (n=58)	Odds ratio (95% CI)	p
Age (years)				
5-9	291	41		
10-14	171	17	0.71 (0.39-1.28)	0.25
Sex				
Male	221	32		
Female	241	26	0.75 (0.43-1.29)	0.29
Filariasis				
Negative	434	57		
Positive	23	1	0.33 (0.04-2.50)	0.29
S. haematobium				
Negative	260	46		
Positive	196	11	0.32 (0.16-0.63)	<0.0001
Continuous variables*				
IgE-total (IU/mL)	691 (246-2372)	767 (281-2262)	1.16 (0.73-1.86)	0.53
IgE-mite (IU/mL)	0.39 (0.15-1.14)	2.28 (0.57-6.00)	6.38 (3.83-10.64)	<0.0001
Histamine wheal (mm)	5.0 (4.5-5.75)	5.4 (4.25-6.00)	1.04 (0.79-1.37)	0.78

Odds ratios of IgE-mite and IgE-total are based on log-transformed data. *Data are median (IQR).

Table 1: **Distribution of skin-test reactivity to mite with odds ratios and 95% CIs according to age, sex, tissue helminth infections, total IgE antibodies, and skin-test histamine wheal size in 520 schoolchildren surveyed in the Lambaréné area**

children (n=215) were examined by two physicians who looked for skin disorders on arms, legs, face, and back attributable to allergy, but only other skin disorders such as heat rash (33 [15%]), scabies (12 [6%]), tinea capitis (nine [4%]), and pityriasis versicolor (seven [3%]) were found. The children's bodyweight and concentrations of haemoglobin were used as indicators of general physical status. Of the 132 children, one physician tested 69, of whom 24 were skin-test reactive to mite, for exercise-induced bronchial hyper-responsiveness. Peak expiratory flows (PEF) were measured before and after running for 10 min, with a portable computerised spirometer. A child was considered to be hyper-responsive if after exercise the PEF had fallen more than 15%.

Cytokine concentrations were measured in the supernatants of cultured peripheral-blood mononuclear cells (PBMC), freshly isolated from heparinised (10 U/mL) venous peripheral blood (20 mL) by density centrifugation over Ficoll (Pharmacia, Sweden).⁸ PBMC at a final concentration of 5×10^6 cells/mL in Iscove's medium (Gibco, UK) containing 10% fetal calf serum were cultured with medium alone or with adult worm antigen of *S. haematobium* (10 µg/mL). Supernatants were collected on day 3 (optimum time for interleukin-10) and day 5 (optimum time for interleukin-5, interleukin-13, and interferon-gamma) and kept at -20°C until transport to Leiden where cytokine concentrations were measured by ELISA. Cytokine ELISA for human interleukin-10, interleukin-13, and interferon-gamma were done with commercial kits (CLB, Netherlands) with detection limits of 3.0 pg/mL, 1.5 pg/mL and 2.0 pg/mL, respectively. For the interleukin-5 ELISA, antibodies TRF5 and JES1-5A10 (Pharmingen, CA) were used as capture and detection antibodies, respectively. As a reference, recombinant human interleukin-5 (Genzyme, MA) was used, with a detection limit of 2 pg/mL.

Statistical analysis

Data on antibodies and cytokines were not normally distributed, so log-transformed data were used in all calculations. We set out to test the hypothesis that helminth infections are negatively associated with atopy, as defined by skin-test reactivity, and to examine whether total IgE antibodies or other immunological factors play

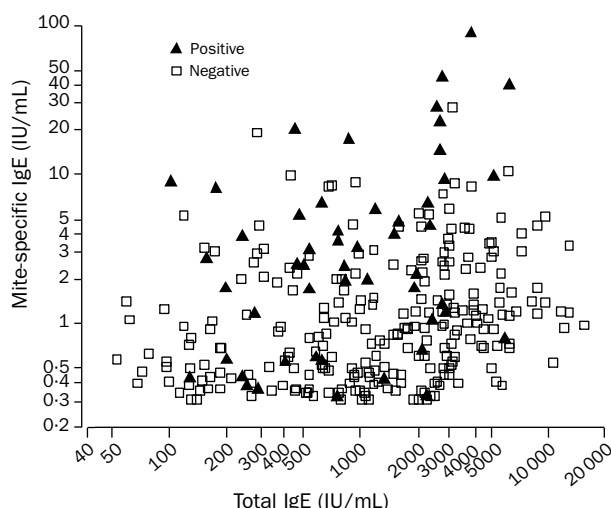


Figure 1: Concentrations of total serum IgE and mite-specific IgE antibodies in 520 children grouped as positive or negative in skin-prick test to mite allergens

an important part in this process. We investigated the association between variables and skin-test reactivity to mite in a univariate logistic-regression model, treating immunological factors as continuous variables. An odds ratio less than 1 indicated a negative association between the variable and the outcome, whereas an odds ratio greater than 1 indicates a positive association. For continuous variables, the odds ratio is a measure of the increase in risk of skin-prick-test mite positivity for a 10-fold increase in the tested variable. An association was considered significant when p was less than 0.05. In the subsample, the same explanatory variables were studied again, with additional immunological data associated with chronic *S haematobium* infections. The association between positivity and infection with *S haematobium* as well as bronchial hyper-responsiveness was calculated by Fisher's exact test. We used the Mann-Whitney test to compare concentrations of specific cytokines produced by different subsets. Variables with a significant association with skin-test reactivity to mite were studied in a multivariate regression to ascertain independent effects of each of the four immunological factors on skin-test reactivity when the other three were taken into account.

Results

The survey showed that the prevalence of skin reactivity to mite, cat, grass, and dog allergens was 11%, 2%, 1%, and less than 0.2%, respectively. Table 1 shows factors that were compared in children with and without atopy as defined by reactivity in skin-prick test. IgE antibodies to mite extract were present in substantial concentrations (>1 IU/mL) in 32% of the studied children and, as expected, higher concentrations of these antibodies were associated with a higher risk of skin reactivity to mite-allergen preparation. Harboring *S haematobium* infection decreased the chance of a positive skin reaction to mite extract by 68%. A similar trend was observed for filarial infections. When we controlled for age, sex, and two indicators of socioeconomic status (the fuel used for cooking and the material houses were constructed from) this did not change the association between infection with *S haematobium* and skin-test reactivity, indicating that these could not have been confounding variables.

Total IgE did not influence the risk of skin reactivity to mite extract. This is also depicted in figure 1, where total

IgE was plotted against mite-specific IgE for all individuals with or without a positive skin test. There was a positive correlation between total IgE and specific IgE ($\rho=0.494$, $p<0.0001$) and the concentrations of total IgE were similar in children with positive and negative skin tests.

Table 2 presents results on the subsample of children; this shows that, as in the larger survey, infection with *S haematobium* was negatively associated with skin-test reactivity to mite. The distribution of age, sex, the material used in the construction of the house, and the fuel used for cooking were not associated with skin-test positivity. Although only a few children were tested for exercise-induced bronchial hyper-responsiveness, two hyper-responsive children were both positive for the skin-prick test to mite.

In children with urinary schistosomiasis, concentrations of interleukin-5 release to adult worm antigen (median 436.4 pg/mL [IQR 40.9–1078.5]), interleukin-10 to adult worm antigen (70.0 pg/mL [26.6–109.4]), and interleukin-13 to adult worm antigen (254.5 pg/mL [66.4–398.0]), were significantly higher ($p<0.0001$ for all three factors) than in those free of infection (interleukin-5 to adult worm antigen 29.3 pg/mL [1.0–151.3], interleukin-10 to adult worm antigen 6.1 pg/mL [1.0–63.4], interleukin-13 to adult worm antigen 31.8 pg/mL [2.4–115.0]). When we tested the association between immunological factors and skin-test reactivity, we found two variables were significantly associated. There was a seven-fold increase in the risk of a positive reaction for each unit increase in concentrations of specific IgE to mite extract and a decrease to 58% for each unit increase in the concentration of interleukin-10 response to adult worm antigen. Two other factors appeared to influence skin

	Negative test (n=102)	Positive test (n=30)	Odds ratio (95% CI)	p
Age (years)				
5–9	61	22		
10–14	41	8	0.54 (0.22–1.33)	0.18
Sex				
Male	58	17		
Female	44	13	0.99 (0.44–2.25)	0.98
Bodyweight (kg)*	27.0 (23.0–32.5)	26.5 (23.0–31.3)	0.97 (0.91–1.03)	0.30
Haemoglobin (g/dL)*	11.9 (10.8–12.8)	11.6 (10.9–12.7)	1.03 (0.80–1.31)	0.83
Material of house				
Wood	39	10		
Stone	55	19	0.74 (0.31–1.77)	0.50
Cooking on gas				
No	58	17		
Yes	36	12	1.14 (0.49–2.66)	0.77
Bronchial hyper-responsiveness				
Negative	45	22		
Positive	0	2		0.12†
<i>S haematobium</i>				
Negative	84	30		
Positive	16	0		0.02†
Immunological factors*				
IgE-mite (IU/mL)	0.38 (0.15–0.91)	2.19 (0.64–5.10)	6.80 (3.01–15.34)	<0.0001
IgE-total (IU/mL)	902 (178–2965)	1976 (494–4126)	1.79 (0.93–3.45)	0.08
IL5-AWA (pg/mL)	51.5 (1.0–232.5)	1.0 (1.0–185.0)	0.72 (0.49–1.03)	0.08
IL10-AWA (pg/mL)	33.2 (1.0–85.2)	1.0 (1.0–37.7)	0.58 (0.36–0.93)	0.02
IL13-AWA (pg/mL)	44.5 (5.0–154.9)	35.8 (7.6–123.6)	0.95 (0.61–1.48)	0.82
IFN-γ-AWA (pg/mL)	1.0 (1.0–133.6)	65.3 (1.0–194.7)	1.17 (0.82–1.66)	0.39

IL=interleukin; AWA=adult worm antigen; IFN=interferon. *Continuous variable, data are median (IQR). † χ^2 analysis with Fisher's exact test.

Table2: Distribution of skin-test reactivity according to demographic data, socioeconomic factors, clinical scores, *S haematobium* infection, and immunological factors in a subset of 132 schoolchildren included in the study of more detailed immunological reactions

	Unadjusted odds ratio (95% CI)	p	Adjusted odds ratio (95% CI)*	p
IgE-mite	6.80 (3.01–15.34)	<0.0001	8.66 (3.16–23.75)	<0.0001
IL5-AWA	0.72 (0.49–1.03)	0.08	0.70 (0.45–1.10)	0.12
IL10-AWA	0.58 (0.36–0.93)	0.02	0.53 (0.30–0.96)	0.04
IgE-total	1.79 (0.93–3.45)	0.08	0.82 (0.35–1.91)	0.64

IL=interleukin; AWA=adult worm antigen. *Adjusted for the IgE mite, IL-AWA, IL10-AWA, and IgE total.

Table 3: Odds ratios and 95% CIs for skin-test reactivity against mite for log-transformed concentrations of four immunological factors in a multivariate model

reactivity but did not reach significance: interleukin-5 release to adult worm antigen and total serum IgE concentrations, the latter associated with a non-significant increased risk of mite reactivity in the skin test (table 2). When the four immunological factors were considered in the same model (table 3), only interleukin-10 to adult worm antigen and mite specific IgE were independently and significantly associated with skin-test positivity (table 3). The values of the odds ratios did not change substantially when controlling for the potential confounding variables age, sex, housing material, and fuel used for cooking.

Discussion

It is clear that high concentrations of IgE are produced to *D pteronyssinus* antigen in the study population, an observation made in other studies in tropical countries.¹⁵ However, we show here that despite this sensitisation, the presence of a chronic tissue helminth infection, urinary schistosomiasis, strongly reduces hypersensitivity to house-dust mite.

Helminths, including schistosomes, stimulate a strong amplification of polyclonal non-specific IgE. Some researchers have argued that the polyclonal nature of this antibody may result in blocking allergic hypersensitivity by either suppressing antigen-specific IgE production or saturating IgE receptors on mast cells and basophils and thereby preventing cellular activation and mediator release by specific antigen.^{15,16} Rigorous statistical analysis here showed that the concentrations of polyclonal IgE did not influence the risk of skin reactivity to mite extract (tables 1 and 3, figure 1). The positive correlation between total IgE and specific IgE also does not support the postulated suppression of allergen-specific IgE by polyclonal antibodies but endorses the studies of Jarrett and colleagues¹⁷ who found that non-specific IgE induced by helminth infections does not inhibit hypersensitivity reactions in vivo.

We show that helminth-induced interleukin-10 lowers the risk of developing skin reactivity to an allergen. Schistosome infection was correlated not only with higher interleukin-10 but also with interleukin-5 and interleukin-13 production in response to adult worm antigen, yet only parasite-induced interleukin-10 was clearly associated with a low risk of skin reactivity. Figure 2 shows the probability of skin-test positivity to mite allergens in children in relation to their concentrations of IgE antibodies to mite antigen and interleukin-10 responsiveness to adult worm antigen. There is a clear suggestion that the effect of IgE on skin-test reactivity is suppressed by the parasite-induced interleukin-10. Anti-inflammatory cytokines such as interleukin-10 and tumour growth factor (TGF- β) are raised in chronic helminth infections and suppress immunological responses.^{11,18} Indeed, helminth infections have also been shown to affect the efficacy of vaccination and impair immune responses to non-parasite vaccine antigens by a mechanism that appears to involve interleukin-10.¹⁹ In

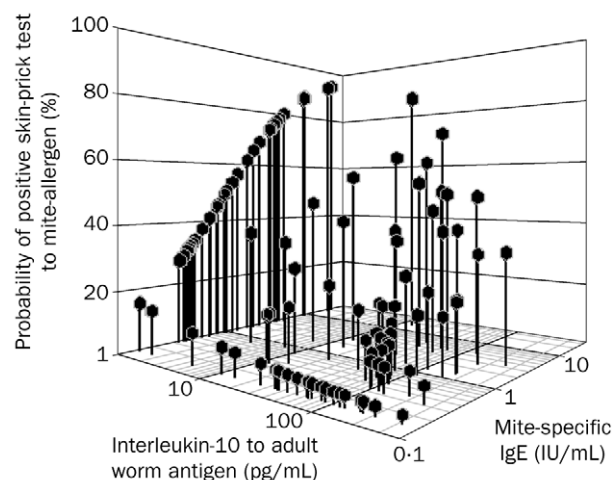


Figure 2: Probability of a positive skin reaction to mite allergens for given concentrations of mite-specific IgE (IU/mL) and interleukin-10 to adult worm antigen (pg/mL).

addition to helminth infections, chronic infections such as tuberculosis and hepatitis are often associated with increased anti-inflammatory cytokine production.^{20,21} In these persistent and chronic infections, relentless antigenic challenge to the immune system evokes strong immunomodulatory responses to control inflammation.²² Regulatory T cells, able to release anti-inflammatory cytokines, may play an important part in immunomodulation.²³ Given a situation in which increased concentrations of anti-inflammatory cytokines are produced, it is also possible that immunological bystander effects result in downregulation of allergic reactions. Thus, in children in developed countries with no extensive experience of chronic infections and a weak immunoregulatory network, exposure to aeroallergens could lead to florid hypersensitivity and inflammatory reactions, precipitating clinical allergy.

The conclusion that interleukin-10 may play a part in counteracting inflammation-mediated allergic diseases is consistent with the fact that in asthmatic patients interleukin-10 production by alveolar macrophages is diminished and production of this cytokine is raised in T and B cells as well as monocytes of patients undergoing immunotherapy. A negative association has also been noted between house-dust-mite wheal size and interleukin-10.^{24–26} Transcription of the interleukin-10 gene is controlled by a polymorphic 5' flanking region, and a study published in 1998 has shown that the haplotype (GCC) associated with low interleukin-10 production is found more frequently in patients with severe asthma.²⁷

Although it is certain that localised Th2 responses, in particular interleukin-13 production, play a crucial part in atopic diseases, it is also clear that additional factors are involved in the fine control of an allergic outcome.²⁸ Genetic factors that may control the degree of Th2-type response or the extent of tissue remodelling can synergise with childhood infections to direct the outcome of exposure to allergens.^{29,30} Our study shows that research into the relation between infectious diseases and atopy should be expanded beyond the Th1/Th2 paradigm. Such investigations should include features that are common to a diversity of chronic infections and negatively associated with the risk of developing atopic diseases. An extensive anti-inflammatory network will need to be considered as a factor that underlies progression to clinical allergy.

Contributors

Anita van den Biggelaar did the field study, immunological measurements, data analysis, and drafted the paper. Ronald van Ree supervised experiments related to allergy and helped to analyse the data and prepare the paper. Laura Rodrigues was responsible for the epidemiological and statistical analysis of the data and helped revise the paper. Bertrand Lell helped with fieldwork and clinical aspects of the study. Andre Deelder advised on parasitological aspects of the study and helped to supervise some immunological measurements. Peter Kremsner helped design the study, supervised the clinical aspects, and was involved in writing the paper. Maria Yazdanbakhsh designed the study, supervised field work and immunological measurements, and revised the paper.

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