Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren

van den Biggelaar, A.H.J.; Rodriques, L.C.; van Ree, R.; van der Zee, J.S.; Hoeksema-Kruize, Y.C.M.; Souverijn, J.H.M.; Missinou, M.A.; Borrmann, S.; Kremsner, P.G.; Yazdanbahsh, M.

DOI
10.1086/381767

Publication date
2004

Published in
The Journal of Infectious Diseases

MAJOR ARTICLE

Long-Term Treatment of Intestinal Helminths Increases Mite Skin-Test Reactivity in Gabonese Schoolchildren

Anita H. J. van den Biggelaar,[1,5,a] Laura C. Rodrigues,[6] Ronald van Ree,[3] Jaring S. van der Zee,[4] Yvonne C. M. Hoeksma-Kruize,[1] John H. M. Souverijn,[2] Michel A. Missinou,[5,7] Steffen Borrmann,[5,7] Peter G. Kremsner,[5,7] and Maria Yazdanbakhsh[1]

Departments of [1]Parasitology and [2]Clinical Chemistry, Leiden University Medical Center, Leiden, and [3]Sanquin Research Department of Allergy, Central Laboratory for Blood Transfusion, and [4]Department of Pulmonology, Academic Medical Center, Amsterdam, The Netherlands; [5]Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon; [6]Department of Infectious Diseases, School of Hygiene and Tropical Medicine, London, United Kingdom; [7]Department of Parasitology, Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany

Background. Several studies have shown an inverse association between helminth infections and atopy, but none have clearly established that the pathogens themselves, rather than other associated factors, cause the suppression of atopy. To show a direct link, prospective intervention studies are required.

Methods. A randomized, controlled trial was performed to study whether repeated anthelminthic treatment results in increased allergic sensitivity to house dust mites (HDMs) in chronically infected children. The trial population consisted of 317 Gabonese schoolchildren with a high prevalence of intestinal helminths. Intervention consisted of treatment every 3 months with praziquantel and mebendazole and with placebo in the control group. Follow-up lasted 30 months: at 6-month intervals, skin-test sensitivity to mites, helminth infection status, and levels of total IgE were determined.

Results. Treatment resulted in a significant increase in the rate of developing skin sensitivity to HDMs (hazard ratio, 2.51; 95% confidence interval, 1.85–3.41), which was mediated, in part, by reductions in *Ascaris* and/or *Trichuris* infections. Levels of total IgE were reduced, but this did not mediate the effect of treatment on skin-test reactivity.

Conclusions. Anthelminthic treatment of chronically infected children results in increased atopic reactivity, which indicates that helminths directly suppress allergic reactions.

Rates of allergic disorders are increasing in the West, and, although allergies are considerably less prevalent in developing countries [1, 2], these conditions are increasing in the progressively urbanizing major cities [3, 4]. Studies have suggested that the reduced exposure to infectious agents that results from successful vaccination programs and the use of antibiotics may underlie the increase in allergic disorders [5, 6]. The immunological explanation for this "hygiene hypothesis" has been that insufficient stimulation of Th1 responses by viruses and bacteria no longer counterbalances the development of Th2 responses and that this allows allergies to develop [7, 8]. However, studies have shown that infections with helminths, which are characterized by strong Th2 responses, are associated with a lower risk of allergic disorders [4, 9, 10]. Helminths might decrease the risk of allergies by stimulating the production of high levels of polyclonal IgE that are capable of blocking Fcε receptors on mast cells [11, 12] or by promoting high levels of regulatory cytokines capable of down-regulating allergic responses [13–15]. The production of parasite-specific interleukin-10 has been shown to be associated with the suppression of atopic reactivity in *Schistosoma haematobium*–infected children in Gabon [16], which suggests that regulatory mechanisms stimulated during chronic helminth infections down-modulate allergic responses [13, 17]. The suppression of atopy by helminth infections seems

Received 3 July 2003; accepted 4 September 2003; electronically published 18 February 2004.

Financial support: Leiden University Medical Center.

[a] Present affiliation: Department of Gerontology, Leiden University Medical Center, Leiden, The Netherlands.

Reprints or correspondence: Dr. Maria Yazdanbakhsh, Dept. of Parasitology, Leiden University Medical Center, Albinusdreef 2, PO Box 9600, 2300 RC Leiden, The Netherlands (m.yazdanbakhsh@lumc.nl).

The Journal of Infectious Diseases 2004; 189:892–900

0022-1899/2004/18905-0018$15.00

to depend on a high burden of parasites—low-level infections, as a result of treatment or low transmission, can stimulate rather than suppress allergic reactivity [18–20], probably by enhancing Th2 responses without sufficiently stimulating the regulatory network [21–24].

Although there is clear evidence of a negative association between helminth infections and atopy, no studies have demonstrated that pathogens themselves, rather than indicating other causal factors, mediate the suppression of atopy. Most studies that have shown an association between helminth infections and atopy had a cross-sectional design. One ecological study showed that, in a group of children, the prevalence of allergic reactivity increased after 2 years of anthelminthic treatment, but the association was at the group, not the individual, level [2]. Prospective—and, preferably, intervention—studies are required to establish a direct link between infectious pathogens and atopy [25, 26]. Because a trial in which humans are intentionally infected with helminths is unlikely to be acceptable, the alternative is to study the effect of removing helminths by treatment. We present the results of a randomized, controlled trial (RCT) of the effect of repeated treatment with anthelminthics on allergic sensitivity to house dust mites (HDMs) in Gabonese schoolchildren.

MATERIALS AND METHODS

Study design. Lambaréné, Gabon, a semiurban municipality with no industrialization that mainly consists of fishing and farming communities in which *Ascaris lumbricoides* and *Trichuris trichiura* are highly prevalent, has been described elsewhere [9, 16, 27]. Skin-test responsiveness is mainly against HDMs (11%), and more than one-third of the children produced substantial levels of mite-specific IgE [9, 16]. The trial study population consisted of 341 children, aged 5–13 years, attending a government school in an area of Lambaréné with a relatively low prevalence of *S. haematobium* (6% of the study population) and filarial worms (*Loa loa* and *Mansonella perstans*), for which none of the children were found to test positive.

Intervention and randomization. The study was an open-label RCT. The treatment regimen was 40 mg/kg body weight of praziquantel and 400 mg of mebendazole to control infections with schistosoma and intestinal helminths, respectively; the control group received a placebo. Randomization was based on blocks of 10 children, with 5 children allocated to receive treatment and 5 allocated to receive placebo, according to an earlier blindly determined fixed sequence, in the order of the sequential appearance of children in the investigator's room, classroom by classroom, and not according to a rule. The original protocol stipulated that the intervention treatment be given every 2 months, but, for logistical reasons (school vacations), this was changed to every 3 months after the first treatment. Drug intake was observed, to monitor compliance and to provide medical supervision, except during vacations, when the pills were given to the parents with instructions on when the children should take them and where to report in case of adverse reactions.

Outcomes. The main objective of the trial was to study whether atopic responses, as measured by skin-test responsiveness, are related to worm infections. This was investigated by examining the effect of repeated anthelminth treatment on conversion to mite skin-test reactivity. Secondary objectives were to explore whether any effect of treatment on skin reactivity was mediated by worm clearance *(A. lumbricoides* and *T. trichiura)* or by changes in the level of total IgE.

Skin-prick testing. Skin tests were performed in all children at entry and every 6 months, using mite extract (*Dermatophagoides pteronyssinus;* HAL Allergen Laboratories), histamine chloride (10 g/L) as a positive control, and allergen diluent as a negative control. Only 1 batch of allergen and controls was used throughout the study. All skin tests were performed by 1 observer. The size of the response was recorded as half the sum of the longest wheal diameter and the diameter perpendicular to it. Skin-test results for HDMs were considered to be positive when children had a negative saline control, the positive histamine response was at least 3 mm, and the mean wheal size of the specific skin test was at least 3 mm [28]. During the study, no positive responses to the negative saline control were recorded. No differences in wheal sizes to the positive histamine control were found between the control and treatment groups during the follow-up period of the study.

Helminth infections. *A. lumbricoides* and *T. trichiura* infections were determined in a subsample of children by the collection and examination of stool every 6 months, always 3 months after the last treatment and before the next dose was given. The number of children with stool-examination results was not the same at all time points and depended on the cooperation of the children in providing stool samples. Numbers were, for treatment and control groups (number who provided a stool sample/total number of children in the group), respectively, 24/152 and 23/165 at time 0; 47/149 and 41/158 at 6 months; 80/124 and 83/149 at 12 months; 50/88 and 44/111 at 18 months; 37/80 and 42/96 at 24 months; and 28/56 and 55/64 at 30 months. Positive diagnosis was based on the presence of eggs in at least 1 of 2 smears of 25 mg of feces, prepared by the Kato Katz method. The presence of *S. haematobium* was determined by detecting eggs in 10 mL of filtered urine in all children at 0, 18, and 30 months.

Levels of total and mite-specific IgE. Total IgE was measured in all children at 6-month intervals in blood drops collected by finger prick on filter paper, using ELISA with rabbit anti–human IgE antibodies (Dako) and goat anti–human IgE biotinylated antibodies (Vector) as capture and detection an-

tibodies, respectively [16]. The World Health Organization standard of human serum IgE was used as a reference (National Institute for Biological Standards and Control). The dilution factor of serum eluted from the filter papers was estimated to be 1:20, which was confirmed by determining albumin levels in the eluates, according to the immunoturbidimetric method of Hitachi for the determination of microalbumin in serum, with the fully automated Hitachi 9111 analyzer.

Serum mite-specific IgE antibodies were measured by radioallergosorbent test, as described elsewhere [29], with Sepharose-coupled *D. pteronyssinus* allergens and radiolabeled sheep antibodies directed to human IgE, in all children at time 0, in 55 children at 12 months, and in all children at 30 months. As a reference, a dilution series of chimeric monoclonal IgE antibody against the major HDM allergen Der p 2 was used. For both IgE assays, results were expressed in international units per milliliter, with 1 IU corresponding to 2.4 ng of IgE. The detection limit of the assay was 0.1 IU/mL, and a value of 0.05 IU/mL was assigned to negative samples.

Ethical considerations. Parents and children were informed of the design and aims of the study during a meeting at the school. Written, informed consent was obtained from parents, and assent was obtained from children. The study was approved by ethics committees of the Medical Faculty of the University of Tübingen, Germany, and the International Foundation of the Albert Schweitzer Hospital in Lambaréné, Gabon. Local clinical practice is to treat helminth infections when they are diagnosed. Ethical clearance not to treat the helminth-infected children in the control group was given because most children never develop clinical disease as a result of infection (all were guaranteed treatment by the trial in the case of symptoms) [30]. Understanding the causes of the increase in allergic diseases in developed countries and in urban centers in developing countries may enable children in the developing world to benefit from a developed lifestyle without paying the price of the increased frequency of atopic diseases.

Statistical methods. The significance of differences in prevalence between groups was tested using the χ^2 test. Because levels of mite-specific and total IgE antibodies and egg counts were not normally distributed, data are expressed as medians with interquartile ranges (IQRs). Differences between groups were tested by nonparametric Mann-Whitney *U* tests. In the Cox regression models, the variables age and total- and mite-specific IgE were used as categories.

Linear mixed models were used to assess whether the treatment and control groups differed significantly in changes over time in continuous variables, such as level of total IgE, mite-specific IgE, and histamine responses. The associations of the variables treatment, time, and the interaction between the 2 were studied in each model, with the interaction between time and treatment representing the longitudinal effect of treatment per 6 months of follow-up.

Survival analysis (Cox regression), which has the advantage of taking into account the time until the event and of allowing inclusion of subjects until they are lost to follow-up, was used to estimate the risk to convert within 30 months in children in the treatment and control groups who had negative skin-test results at the start of the trial. Children who converted to a positive skin-test result were excluded from follow-up and were not reintroduced if their skin-test results reverted to negative. To explore the causal mechanisms of the effect of treatment on skin-test conversion, 2 possibly mediating variables (helminth infection and level of total IgE) were added, 1 at a time, to the basic model (controlling for age, sex, and mite-specific IgE). In such a hierarchical approach, inclusion in the basic model of an intermediate proximal variable in a chain of causation reduces the magnitude of association (the value of the hazard ratio [HR]) between the distal variable (treatment) and the outcome (skin reactivity). Both helminth infection and level of total IgE were treated as time-dependent variables. Children with missing values for stool examination at each time point were excluded from the analyses at that point. HRs were obtained by Cox regression, with $HR > 1$ indicating an increased risk and $HR < 1$ indicating a reduced risk. An association was considered to be significant at $P < .05$.

RESULTS

Study population. Figure 1 presents the flow through the trial of the 317 skin-test–negative children. A total of 152 children were lost to follow-up (83 in the control group and 69 in the treatment group; $P = .26$), mostly because they left school and could not be traced; only 6 children refused to continue the study. Migration is a well-known phenomenon in Gabon, and children often move to live with other family members for undefined periods of time. Parents usually wait for the school year to end to migrate out of the area; therefore, the loss to follow-up was particularly high after the school holidays. Children started treatment in May 1998, but, for logistical reasons, 1 group of children ($n = 35$) started treatment in November 1998 and was followed for only 24 months, which contributes to the apparently large lost-to-follow-up value between 24 and 30 months.

At baseline, 46% of the children were estimated to be infected with *A. lumbricoides* and 71% with *T. trichiura,* with median egg loads for *Ascaris* of 25,560 eggs/g (IQR, 2080-63,980 eggs/g) and for *Trichuris* of 1240 eggs/g (IQR, 440-3280 eggs/g). The 152 children allocated to the treatment group and the 165 children allocated to the control group were equivalent at the onset of the study, with respect to age, sex, levels of mite-specific and total IgE, and size of skin-test responses to histamine (table

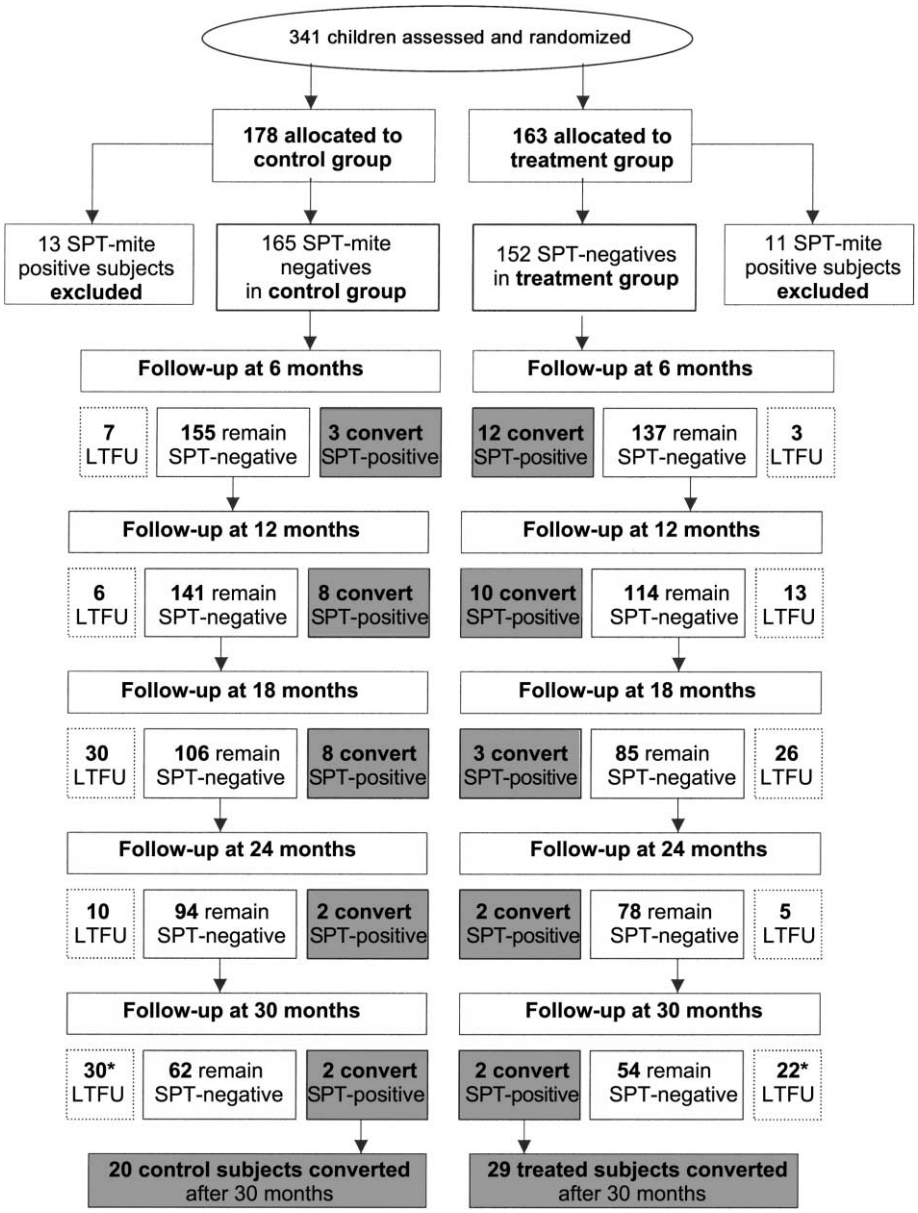

Figure 1. Flow diagram showing the number of recruited, randomized, and followed children in the study. For each 6-month follow-up period, the no. of children who converted to a positive skin-prick test (SPT) result for mites, who were lost to follow-up (LTFU), or who continued in the follow-up study are indicated. Because 35 children started treatment 6 months later, of which 18 children completed a follow-up period of 24 months, the no. lost to follow-up was relatively high at 24 months (*).

1). Children lost to follow-up, in either the treatment or the control group, did not differ from children who completed the study, in terms of baseline characteristics (data not shown). In total, 11 children were infected with *S. haematobium* in the control group and 7 in the treatment group ($P = .45$).

The effect of treatment on skin-test reactivity to HDMs. In both the treatment and control groups, children converted to a positive skin-test result for HDMs ($n = 29$ in the treatment group and $n = 20$ in the control group), and most children who converted also reversed during the trial (20/29 and 15/20 in the treatment and control groups, respectively; $P = .65$). The cumulative proportion of children converting to a positive skin-test result for HDMs during the 30-month study period was significantly higher in the treatment group than in the control group (HR of skin-test result conversion associated with treatment, 1.77; 95% confidence interval [CI], 1.40–2.23) (figure 2). Because sex was found to modify the effect of treatment on skin-test conversion ($P = .06$ for interaction), Cox regression analysis was performed for girls and boys separately, and girls were found to have a significantly higher risk of treatment-

Table 1. Baseline measurements in control and treatment groups of children with helminth infections.

Characteristic	Group		*P*
	Control (*n* = 165)	Treatment (*n* = 152)	
Age, years			
5–9	123	111	
10–13	42	41	.705
Sex, no.			
Female	84	74	
Male	81	78	.691
Total IgE, median (IQR), IU/mL	535 (174–2188)	471 (200–2291)	.850
Mite-specific IgE, median (IQR), IU/mL	0.38 (0.20–1.15)	0.52 (0.26–1.82)	.138
Histamine, median (IQR), mm	5.00 (4.50–5.75)	5.00 (4.50–5.75)	.364
Ascaris, median (IQR), eggs/g	20,140 (13460–60,940)	14,520 (3600–50,120)	.209
Trichuris, median (IQR), eggs/g	500 (20–1780)	470 (20–3280)	.940

NOTE. The distribution of variables determined at the time of recruitment was compared for children randomly allocated to the treatment or control group. *P* values are based on χ^2 tests and Mann-Whitney *U* tests for comparison of prevalence and continuous data, respectively. IQR, interquartile range.

induced conversion (HR, 2.19; 95% CI, 1.58–3.02) than boys (HR, 1.44; 95% CI, 1.03–2.02).

The response to the positive histamine control was found to increase slightly over time (0.12 ± 0.03 mm/6 months of follow-up; $P < .001$) in the total study population, with no difference between the control and treatment groups ($P = .718$ for interaction), which indicates that nonspecific effects of treatment on skin-test responses can be ruled out. No child responded to the negative saline control during the total study period.

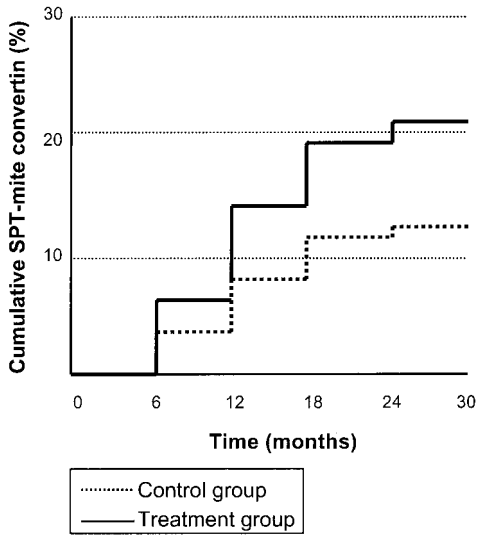

Figure 2. Cumulative conversion rate of study children during 30 months. Children with negative skin-prick test (SPT) results in both the treatment and control groups were skin tested at 6-month intervals for house dust mites. Comparable to a "1 − survival" plot, the graph shows a "1 − remaining skin-test negative" plot. In the treatment group, relatively more children converted to a positive mite skin test during 30 months than in the control group (hazard ratio, 1.77; 95% confidence interval, 1.40–2.23).

Effect of treatment on helminths, mite-specific IgE, and total IgE. From 6 months onward, significantly fewer infections with *A. lumbricoides* and/or *T. trichiura* were diagnosed in the treatment group than in the control group ($P = .002$), with no *A. lumbricoides* eggs seen in the treatment group by the end of the trial (figure 3*A*–3*C*). Although infections with *T. trichiura* remained present in treated children, the intensity of infection was significantly reduced during the course of treatment (estimated change, −122.8 ± 5.8 eggs/g/6 months; $P < .001$). Supporting the results of skin-prick test (SPT) conversion, treatment was found to be less effective in boys than in girls (after 30 months of treatment, 25% of the boys remained infected with *Ascaris* and/or *Trichuris* vs. 7% of the girls, despite infection prevalence being similar at the beginning of the study). In the control group, the prevalence of intestinal helminth infections remained unchanged during the trial (figure 3*A*–3*C*).

In the control group, median levels of mite-specific IgE were 0.38 IU/mL (IQR, 0.20–1.15 IU/mL) at entry, 0.34 IU/mL (IQR, 0.08–2.07 IU/mL) at 12 months, and 0.20 IU/mL (IQR, 0.08–0.50 IU/mL) at 30 months. In the treatment group, median levels of mite-specific IgE were 0.52 IU/mL (IQR, 0.26–1.82 IU/mL) at entry, 0.62 IU/mL (IQR, 0.18–1.82 IU/mL) at 12 months, and 0.26 IU/mL (IQR, 0.11–2.21 IU/mL) at 30 months, but statistical analysis by mixed linear models showed that there was no significant difference in levels mite-specific IgE in time ($P = .974$) or between the treatment and control groups ($P = .790$ for interaction). Levels of total IgE antibodies declined significantly during the follow-up period (estimate, −138 ± 50 IU/mL/6

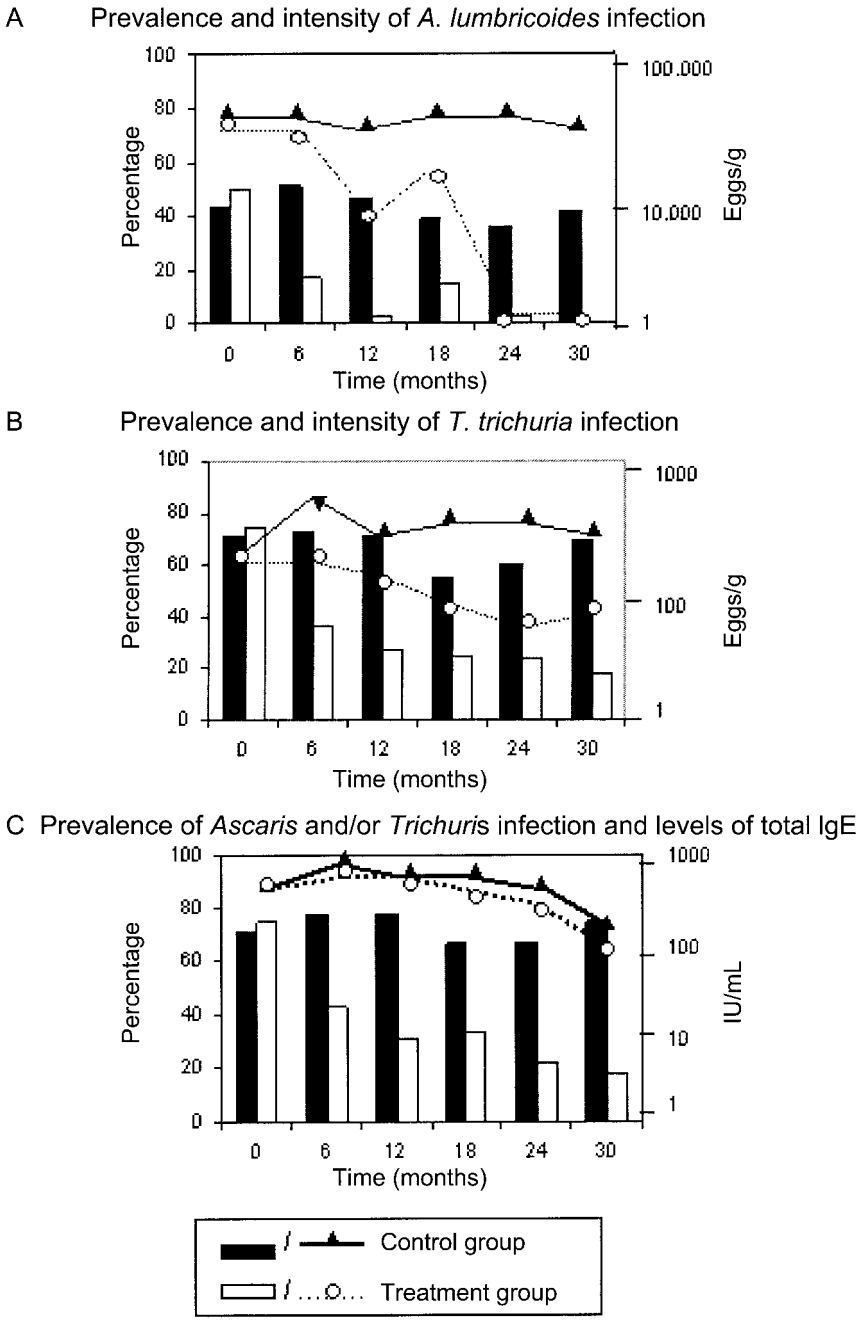

Figure 3. Effect of treatment on helminth infection and levels of total IgE. Children were screened for infections with *Ascaris lumbricoides* and *Trichuris trichiura* at 6-month intervals. The no. of children with stool-examination results were not the same at all time points and depended on the cooperation of the children. Nos. were, respectively, for treatment and control groups, 24/152 and 23/165 at time 0; 47/149 and 41/158 at 6 months; 80/124 and 83/149 at 12 months; 50/88 and 44/111 at 18 months; 37/80 and 42/96 at 24 months; and 28/56 and 55/64 at 30 months. At the onset of the study, the prevalence of infection *(A–C)* was similar for children in the treatment and control groups, but, from 6 months of treatment onward, the infection prevalence was significantly lower in the treatment than in the control group ($P < .01$). *A,* Treatment resulted in a complete clearance of *Ascaris* infections after 30 months. *B,* Although *Trichuris* infections persisted in some treated children, the intensity of infection was significantly reduced. *C,* During the 30 months of follow-up, levels of total IgE decreased in both control and treated children, but the decline was more marked in the treated group.

months; $P = .006$), with a significantly higher decrease in the treatment group than in the control group (estimate, -136 ± 68 IU/mL/6 months; $P = .045$ for interaction).

Independent effect of helminth infection and levels of total IgE on skin-test reactivity. Cox regression analysis showed that the relative risk to convert in infected children was almost half of that in noninfected children (HR, 0.56; 95% CI, 0.35–0.90), which demonstrates a significant suppressive effect of helminth infection on skin-test reactivity. The possible effect of levels of total IgE on skin-test reactivity was studied by Cox regression: nonsignificant relative risks of 1.15 (95% CI, 0.69–1.90) and 1.62 (95% CI, 0.95–2.75) were found for children who produced total IgE levels of 100–1000 or >1000 IU/mL, respectively, compared with children who produced <100 IU/mL (reference group), which indicates that the risk to convert was not associated with levels of total IgE.

Changes in* Ascaris *and* Trichuris *levels mediate treatment-induced skin-test conversion. To explore what mediated the effect of treatment, we first defined a basic model (model 1) that controlled for confounding with age, sex, and levels of mite-specific IgE. In this model, the significant adjusted effect of treatment on skin-test reactivity was 2.51 (95% CI, 1.85–3.41) (table 2). When helminth infection was added to the basic model (model 2), the effect of treatment was reduced to 2.02 (95% CI, 1.19–3.43). Treated children who had cleared their infection had a significant (3.5 times higher) risk to convert than children who, despite treatment, remained infected (95% CI, 1.37–8.95; $P = .009$). When the level of total IgE was introduced into the basic model (model 3), there was no reduction in the effect of treatment (table 2).

DISCUSSION

We have described an RCT that demonstrated an increase in allergic sensitivity with treatment of helminth infections at the individual level and thus provided evidence for the direct suppressive role of helminth infections on atopic reactions. The finding that, independent of treatment, children free of infection had twice the risk to convert, relative to infected children, confirms the role of helminths in atopy. Although compliance bias is very unlikely and there is no other clear explanation or consistent data in the literature for the observed difference in the efficacy of anthelminthic treatment in boys and girls, the stronger SPT conversion in girls is consistent with the higher reduction in intestinal helminth infections that is seen in girls than in boys. The association between treatment and skin-test conversion appeared to be mediated only in part by the clearance of helminth infections: this may reflect an imperfect measurement of the helminth infections caused by the possible less-effective clearance and presence of parasites other than *Ascaris* and *Trichuris* species (e.g., hookworm or strongyloides [31]) or a change in the relationship between worm burden and egg output after chemotherapy [32]. Although we cannot rule out a direct effect of the treatment drugs on allergic sensitivity, treatment was not found to affect the wheal sizes to histamine control. The IgE antibodies to mites, measured in a subsample of children during follow-up, did not change during treatment, which indicates that the effect of treatment on skin-test reactivity is unlikely to be mediated by mite-specific IgE.

Two mechanisms have been postulated for the negative association between helminth infections and allergic diseases: (1) the blocking of Fcε receptors by high levels of helminth-stimulated polyclonal IgE [11, 12] and (2) the suppression of allergic responses by a helminth-induced regulatory network [13]. Our results show clearly that, at least in this setting, although levels of total IgE started to decrease 18 months after treatment, the effect of treatment on increased skin-test reactivity was not mediated by changes in levels of total IgE, which corresponds with the results of earlier cross-sectional studies [4, 9, 16].

The conversion in skin-test reactivity appeared to be a nonpersisting event. This temporary nature of the increased sensitivity after treatment might reflect a temporary alleviation of the responses that regulate or suppress atopic reactivity [9, 33]. In the absence of reinfection, the clearance of chronic helminth infection results in a progressive loss of immune suppression, as was shown in a study of chronically infected Ethiopians

Table 2. Risk of skin-prick test conversion: Cox regression of the causal pathway of the treatment-induced risk to convert to mite skin-test reactivity.

Model	HR (95% CI)	*P*
1, adjusted for age, sex, and levels of mite-specific IgE	2.51 (1.85–3.41)	<.001
2, adjusted for age, sex, levels of mite-specific IgE, and infection status	2.02 (1.19–3.43)	.010
3, adjusted for age, sex, and levels of mite-specific and total IgE	2.50 (1.80–3.47)	<.001

NOTE. To explore the causal pathway of the treatment-induced risk to convert, its association with treatment was studied in several models of multiple Cox regression. First, in a basic model, the association of treatment with the risk to convert was studied while adjusting for age, sex and levels of mite-specific IgE. Next, the influence of infection status and levels of total IgE on the association in the basic model was studied by adding these variables stepwise to the model. When an added variable is more proximal in the chain of causation, the association of treatment with conversion is expected to be reduced. CI, confidence interval; HR, hazard ratio.

treated with anthelminthics after their migration to an area in Israel where helminth infections were not endemic [34]. Reinfection is expected to be high in our study area and, for each child, will occur to a varying extent and, at varying times, between 2 treatment periods. The presence of juvenile worms, which are not yet capable of producing eggs, may boost the immune system sufficiently to restore the regulatory responses and suppress atopy. Our results show that populations living in areas where helminth infections are endemic, which often show a low prevalence of allergic disorders, have the potential to mount atopic immune responses when the suppression induced by chronic and high burdens of infection is removed. Although we did not study clinical allergy, the finding that atopic reactivity was increased by a moderate degree of parasite clearance raises concerns regarding the development of allergic diseases in progressively "dewormed" populations. The importance and priority of eradicating infectious diseases in low-income countries is beyond dispute, but understanding the mechanisms by which infectious pathogens down-regulate allergic responses may enable the identification of new interventions for the optimal eradication of infectious diseases, while leaving immunoregulatory mechanisms unchanged, and inform new research approaches to control atopic diseases.

Acknowledgments

We thank Rob Aalberse and Paolo Matricardi for critically reading the manuscript and Phaedon Ellinas (Medochemie, Ltd., Limassol, Cyprus) for the gift of Praziquantal.

References

1. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet **1998**; 351:1225–32.
2. Lynch NR, Hagel I, Perez M, Di Prisco MC, Lopez R, Alvarez N. Effect of anthelminthic treatment on the allergic reactivity of children in a tropical slum. J Allergy Clin Immunol **1993**; 92:404–11.
3. Yemaneberhan H, Bekele Z, Venn A, Lewis S, Parry E, Britton J. Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia. Lancet **1997**; 350:85–90.
4. Scrivener S, Yemaneberhan H, Zebenigus M, et al. Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. Lancet **2001**; 358: 1493–9.
5. Matricardi PM, Rosmini F, Ferrigno L, et al. Cross-sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. BMJ **1997**; 314:999–1003.
6. Alm JS, Swartz J, Lilja G, Scheynius A, Pershagen G. Atopy in children of families with an anthroposophic lifestyle. Lancet **1999**; 353:1485–8.
7. Cookson WO, Mofatt MF. Asthma: an epidemic in the absence of infection? Science **1997**; 275:41–2.
8. Holt PG, Sly PD. Interactions between respiratory tract infections and atopy in the aetiology of asthma. Eur Respir J **2002**; 19:538–45.
9. van den Biggelaar AHJ, van Ree R, Rodrigues LC, et al. Decreased atopy in children infected with *Schistosoma haematobium:* a role for parasite-induced interleukin-10. Lancet **2000**; 356:1723–7.
10. Hagel I, Lynch NR, DiPrisco MC, Lopez RI, Garcia NM. Allergic reactivity of children of different socioeconomic levels in tropical populations. Int Arch Allergy Immunol **1993**; 101:209–14.
11. Godfrey RC, Gradidge CF. Allergic sensitisation of human lung fragments prevented by saturation of IgE binding sites. Nature **1976**; 259: 484–6.
12. Hagel I, Lynch NR, Perez M, Di Prisco MC, Lopez R, Rojas E. Modulation of the allergic reactivity of slum children by helminth infection. Parasite Immunol **1993**; 15:311–5.
13. Yazdanbakhsh M, van den Biggelaar A, Maizels RM. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol **2001**; 22:372–7.
14. Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Immunol **2001**; 1:69–74.
15. King CL, Medhat A, Malhotra I, et al. Cytokine control of parasite-specific anergy in human urinary schistosomiasis: IL-10 modulates lymphocyte reactivity. J Immunol **1996**; 156:4715–21.
16. van den Biggelaar AHJ, Lopuhaa C, van Ree R, et al. The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. Int Arch Allergy Immunol **2001**; 126:231–8.
17. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites and the hygiene hypothesis. Science **2002**; 296:490–4.
18. Lynch NR, Hagel IA, Palenque ME, et al. Relationship between helminth infection and IgE response in atopic and nonatopic children in a tropical environment. J Allergy Clin Immunol **1998**; 101:217–21.
19. Buijs J, Borsboom G, Renting M, et al. Relationship between allergic manifestations and *Toxocara* seropositivity: a cross-sectional study among elementary school children. Eur Respir J **1997**; 10:1467–75.
20. Palmer LJ, Celedon JC, Weiss ST, Wang B, Fang Z, Xu X. *Ascaris lumbricoides* infection is associated with increased risk of childhood asthma and atopy in rural China. Am J Respir Crit Care Med **2002**; 165:1489–93.
21. Cooper PJ, Mancero T, Espinel M, et al. Early human infection with *Onchocerca volvulus* is associated with an enhanced parasite-specific cellular immune response. J Infect Dis **2001**; 183:1662–8.
22. Holland MJ, Harcus MY, Riches PL, Maizels RM. Proteins secreted by the parasitic nematode *Nippostrongylus brasiliensis* act as adjuvants for Th2 responses. Eur J Immunol **2000**; 30:1977–87.
23. Doetze A, Satoguina J, Burchard G, et al. Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by T(h)3/T(r)1-type cytokines IL-10 and transforming growth factor–β but not by a T(h)1 to T(h)2 shift. Int Immunol **2000**; 12:623–30.
24. Paterson JC, Garside P, Kennedy MW, Lawrence CE. Modulation of a heterologous immune response by the products of *Ascaris suum*. Infect Immun **2002**; 70:6058–67.
25. Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet **2001**; 357:1076–9.
26. Kalliomäki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Poibiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet **2003**; 361:1869–71.
27. Montresor A, Crompton DWT, Bundy DAP, Hall A, Savioli L. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community levels [WHO/CTD/SIP/98.1]. Geneva: World Health Organization, **1998**.
28. Dreborg S, Frew A. Allergen standardisation and skin tests. EAACI position paper. Allergy **1993**; 48:49–82.
29. Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and *Hymenoptera* venom. J Allergy Clin Immunol **1981**; 68:356–64.
30. Chan MS, Medley GF, Jamison D, Bundy DA. The evaluation of potential global morbidity attributable to intestinal nematode infections. Parasitology **1994**; 109:373–87.

31. Musgrave IA, Hawes RB, Jameson JL, Sloane RA, Quayle PA. Evaluation of a new antihelminthic for trichiurasis, hookworm, and strongyloidiasis. Med J Aust **1979**; 1:403–5.
32. Holland CV, Asaolu SO, Crompton DWT, Stoddart RC, Macdonald R, Torimiro SEA. The epidemiology of *Ascaris lumbricoides* and other soil-transmitted helminths in primary school children from Ife-Ife, Nigeria. Parasitology **1989**; 99:275–85.
33. Grogan JL, Kremsner PG, Deelder AM, Yazdanbakhsh M. Elevated proliferation and interleukin-4 release from $CD4^+$ cells after chemotherapy in human *Schistosoma haematobium* infection. Eur J Immunol **1996**; 26:1365–70.
34. Borkow G, Leng Q, Weisman Z, et al. Chronic immune activation associated with intestinal helminth infections results in impaired signal transduction and anergy. J Clin Invest **2000**; 106:1053–60.