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The effect of three-monthly albendazole treatment on Th2 responses: Differential effects on IgE and IL-5

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Abstract

Helminth parasites induce a strong Th2 response, characterized by high levels of IgE and elevated signature cytokines such as IL-5. As many global deworming programs are underway, there is concern that this might lead to emergence of Th1-mediated pathologies when the counterbalancing helminth-induced Th2 response is absent. Therefore, we assessed the effect of deworming on Th2 mediated responses in a household-clustered randomised controlled trial in Indonesia.

Total plasma IgE and whole-blood IL-5 responses to mitogen phytohaemagglutinin (PHA) were measured in 1494 and 682 subjects respectively, at baseline, 9 and 21 months after three-monthly single dose treatment with albendazole or placebo.

Anthelmintic treatment did not result in complete removal of helminth infections in the community. However, treatment significantly decreased IgE levels in albendazole compared to placebo-treated subjects. IL-5 responses to PHA were not significantly affected by anthelmintic treatment and tended

to increase in albendazole-treated subjects, indicating that intensive treatment of helminth parasites has different outcomes on B cell (IgE levels) and T cell (IL-5) responses. The data shows that two years of deworming can have differential effects on responses typified as Th2 mediated, which needs to be taken into account when examining the impact of helminths on non-communicable diseases.

Keywords: helminths, albendazole, Th2, IgE, IL-5, Indonesia

Introduction

Amongst helminth parasites, soil-transmitted helminths (STH) which reside in the gut, are most prevalent. More than 1.5 billion people are infected with STH worldwide and infections are widely distributed in tropical and subtropical countries (1). Important STH include *Ascaris lumbricoides*, *Trichiuris trichiura* and hookworm (*Ancylostoma duodenale* and *Necator americanus*).

Helminth parasites are the most potent natural stimuli for T-helper 2 (Th2) responses, characterized by Th2 cytokines (IL-4, IL-5 and IL-13), eosinophilia and high levels of IgE antibodies. Whereas IL-5 regulates eosinophilia, IL-4 and IL-13 promote IgE production by inducing B cell switching to IgE-producing cells.

Th2 responses do not solely protect against helminth infections but also play an important role in the development of various inflammatory diseases, where an imbalance between Th1/Th2 cell responses and their regulation can lead to pathogenesis. Studies in animal models and in humans have indicated that helminths might play a protective role in the development of allergies, autoimmunities and inflammatory bowel disease (2). Moreover, recent *in vivo* studies showed that helminth infections might protect against insulin resistance as helminth-induced Th2 responses were associated with improved glucose metabolism and insulin signalling in mice (3-6).

This has raised concern regarding the implementation of deworming programs, which might lead to emergence of Th1-mediated pathologies. The immunological consequences of deworming should therefore be examined in detail, given that Th2 responses consist of multiple arms.

To this end, we analysed the immunological data obtained from a household-based cluster randomized double blind placebo-controlled trial of three-monthly single dose albendazole treatment in an area where STH are highly endemic (7). This study describes the effect of anthelmintic treatment on two different components of the Th2 response, namely total IgE levels and total IL-5 production in response to a polyclonal stimulation (phytohemagglutinin, PHA).

Materials and Methods

Study design

This report describes a nested study within the ImmunoSPIN trial (8, 9). The trial was conducted in two villages, Nangapanda and Anaranda, in Ende district, Flores island, Indonesia. In 2008 the double blind placebo-controlled trial of two year duration was initiated by randomizing all households to receive either a single dose of 400 mg albendazole or a matching placebo every three-months over a two year study period (tablets from PT Indofarma Pharmaceutical, Bandung, Indonesia). Treatment allocation was based on household to minimise the risk of cross contamination and therefore reinfection of treated individuals. Treatment was provided to all household members older than two years of age, except for pregnant women. Drug intake was observed by field workers. The study was approved by the Ethical Committee of the Medical Faculty, University of Indonesia, Jakarta (ref: 194/PT02.FK/Etik/2006) and has been filed by the ethics committee of the Leiden University Medical Center, The Netherlands. The trial was registered as clinical trial (ISRCTN83830814). Informed consent or parental consent was obtained from all participants.

Study population

The randomization for the total study was based on 954 households comprising of 4004 individuals in the two villages, resulting in 2022 (481 houses) and 1982 (473 houses) subjects in the placebo and albendazole group, respectively, as described before (7). The current study included 1762 (363 houses) and 1731 (362 houses) subjects in the placebo and albendazole group in the village of Nangapanda, respectively (figure S1). At baseline, blood samples from 2349 subjects were collected to analyse total IgE levels and after inclusion of subjects with at least one follow-up blood sample, 1494 subjects were included at baseline, corresponding to 753 placebo- and 741 albendazole-treated individuals.

To study immunological responses, 250 households were randomly selected and individuals older than 4 years of age were invited for morning venous blood sampling and assessment of anthropometric parameters. This resulted in the inclusion of 882 individuals, of which 858 provided sufficient blood samples for whole blood cultures. After inclusion of subjects with at least baseline and one follow-up blood sample, we ended up with 682 subjects at baseline, corresponding to 374 placebo- and 308 albendazole-treated individuals (figure S1). As this is a nested immunology study, 86% (584/682) of the subjects were also part of the group of 1494 subjects included for the analysis of IgE levels.

Parasitological examination

Yearly stool samples were collected in order to examine the effect of treatment on helminth prevalence. *T. trichiura* was detected by microscopy after formol-ether concentration and 18S-based multiplex real-time PCR was used for the specific amplification and detection of hookworm (*A. duodenale, N. americanus), A. lumbricoides,* and *S. stercoralis* DNA, as described previously (9). PCR output was expressed as the cycle threshold (Ct) reflecting the load of parasite specific DNA in the sample tested. Parasite specific DNA loads of *A. duodenale*, *N.americanus* and *A.lumbricoides* were categorized as low load (Ct≥30) and high load (Ct<30), irrespective of infection with *T. trichiura* of which only microscopy data were available. A subject was defined to have a heavy infection when a high DNA load (Ct<30) was measured for either hookworm or *A. lumbricoides*. Whereas a light infection refers to subjects with low DNA loads (Ct≥30) for both of these helminth species, including subjects that are helminth negative.

Plasma IgE

The levels of total IgE were measured by ELISA in Jakarta as described previously (9). The results are expressed in International Units (IU/ml).

Whole blood culture and IL-5 measurements

Whole-blood was stimulated *in vitro* as described before (9, 10). Cultures were stimulated for 72h to detect adaptive responses to phytohaemagglutinin (PHA, 2 µg/ml, Wellcome Diagnostics, Darford, UK) and unstimulated control wells were included. Due to budget restrictions we could only measure one Th2 cytokine and opted for IL-5. Supernatants were stored at -20°C until IL-5 was quantified using Luminex cytokine kits (Biosource, Camarillo, USA) on a Liquichip 200® Workstation (Qiagen, Venlo, The Netherlands) equipped with Liquichip analyzer software (Qiagen, Venlo, The Netherlands). Cytokine levels that fell below the assay's detectable range were replaced by half of the detection limit provided by the manufacturer.

Statistical analysis

The total IgE and cytokine data were log transformed to obtain a normally distributed variable. Cross-sectional comparisons between groups at baseline were tested with Student's t-test. To assess treatment effects, generalized linear mixed models were used with addition of three random effects, namely a random household-specific intercept to model clustering within households and a random subject-specific intercept and slope to model correlation within subjects, as previously described (10). Parameter estimates for treatment effects at 9 and 21 months and 95% confidence intervals

are reported, as well as interaction p-values when a subgroup analysis was performed. The reported p-values are obtained using likelihood ratio tests by comparing the model with and without the treatment effect. All models were fitted using the lme4 package (11). The analysis was intention-to-treat, and involved all participants as assigned randomly at the start of the trial.

Results

Study population

Baseline characteristics of the study participants are shown in table 1. At baseline 84.8% of the individuals were infected with one or more helminth species, with hookworm infections being the most prevalent (73.1% of total). At baseline total IgE levels and IL-5 production in response to PHA were similar in both treatment arms. The consort diagram of the study is shown in figure S1. Subjects were included in the analysis when data from baseline and at least one of the two follow-up time points were available.

Effect of albendazole treatment on helminth prevalence

Similar to the major study (7), subset analysis in this study revealed that intensive treatment with albendazole resulted in a reduction in STH both after 9 (percentage infected 49.9% for albendazole vs. 80.5% for placebo) and after 21 months of treatment (39.9% for albendazole vs. 75.4% for placebo) (figure 1A). Albendazole had the largest effect on hookworm (from 74.4% at baseline to 29.0% at 9 months and 20.7% at 21 months of treatment) compared to placebo (from 71.8% to 66.7% to 62.1% respectively). This was followed by *A. lumbricoides* (albendazole from 33.3%, to 14.5% and 10.1%; placebo from 35.1% to 33.6% and 32.1%), while the effect on *T. Trichiura* was much less pronounced (albendazole from 27.7%, to 22.0% and 18.2%; placebo from 25.8%, to 30.0% and 25.9%).

Baseline IgE levels

Baseline analysis of total IgE in subjects with light and heavy infections, as defined in Materials and Methods (section parasitological examination), revealed that higher infection intensity was associated with significantly elevated IgE levels (light infection: 809 (695-940) IU/ml [GM, 95 CI], heavy infection: 1544 (1376-1732) IU/ml, $p<0.001$) (table S1). Polyclonal IgE levels were significantly lower in adults (1185 (1086-1294) IU/ml) compared to children ≤ 19 years (1766 (1561-1999) IU/ml) ($p<0.001$), whereas no significant difference was found between males (1307 (1189-1437) IU/ml) and females (1434 (1283-1603) IU/ml) ($p=0.219$).

Effect of albendazole treatment on plasma IgE levels

Figure 1B presents the estimated effect of treatment on total IgE levels after 9 and 21 months. Total IgE levels were significantly lower after 9 and 21 months in the albendazole-treated group (estimates [95% CI] at 9 months -0.071 [-0.112 – -0.030], at 21 months -0.060 [-0.099 – -0.020]; overall p-value: p_{time}<0.001). When infection was defined as light or heavy based on parasite specific DNA loads of hookworm and *A. lumbricoides* at baseline, the estimated treatment effect on IgE levels was significant in subjects with a heavy infection at baseline (at 9 months -0.074 [-0.142 – -0.005], at 21 months -0.083 [-0.146 – -0.019]; p_{time}=0.051). In subjects with light infections we observed no significant treatment effect on IgE (at 9 months -0.039 [-0.126 – 0.047], at 21 months -0.001 [-0.08 – 0.079]; p_{time}=0.664; p-value for interaction=0.252), nor in subjects who were uninfected at baseline (estimates [95% CI] at 9 months -0.134 [-0.280 – 0.012], at 21 months -0.040 [-0.078 – 0.158]; overall p-value: p_{time}= 0.140).

Similar effects of treatment on IgE levels were observed in children (at 9 months -0.069 [-0.134 – 0.004], at 21 months -0.071 [-0.134 – 0.009]; p_{time}=0.003) and adults (at 9 months -0.070 [-0.120 – -0.021], at 21 months -0.053 [-0.099 – -0.007]; p_{time}=0.006; p-value for interaction=0.876). Albendazole treatment reduced total IgE to a similar extent in both males (at 9 months -0.038 [-0.102 – 0.025], at 21 months -0.067 [-0.125 – -0.009]; p_{time}=0.059) and females (at 9 months -0.093 [-0.143 – -0.042], at 21 months -0.055 [-0.103 – -0.007]; p_{time}=0.001; p-value for interaction=0.349).

Baseline IL-5 production in response to PHA

At baseline, we detected no significant difference in IL-5 levels to PHA between subjects with light (474 (242-775) pg/ml [median, IQR]) and heavy infections (490 (283-801) pg/ml) (p=0.364) (table S1). IL-5 responses were significantly lower in adults (472 (258-728) pg/ml) compared to children (534 (319-841) pg/ml) (p=0.04). When considering gender, significantly higher levels of IL-5 were observed in males (588 (347-884) pg/ml) compared to females (460 (259-698) pg/ml) (p<0.001).

Effect of albendazole treatment on whole blood IL-5 responses to PHA

There was no significant treatment effect on IL-5 production in response to PHA at 9 months (estimates [95% CI], 0.037 [-0.052 – 0.127]). However, we observed significantly elevated levels of IL-5 to PHA at 21 months (0.107 [0.000 – 0.214]). When both time points were taken into account, the overall p-value over time fell short of statistical significance (p_{time}=0.146). IL-5 measured in unstimulated blood revealed no treatment-related differences (data not shown).

Additionally, we observed no effect of treatment on IL-5, nor differences in the treatment effect between subgroups when subjects with light and heavy infections (p-value for interaction=0.850) or

children and adults (p-value for interaction=0.998) were analysed separately. In males, treatment did not show a significant effect on the IL-5 response (at 9 months -0.027 [-0.163 – 0.108], at 21 months 0.041 [-0.135 – 0.218]; p_{time}=0.780), whereas a trend towards an increase in IL-5 response was observed in females (at 9 months 0.086 [-0.031 – 0.203], at 21 months 0.164 [0.018 – 0.311]; p_{time}=0.192; p-value for interaction=0.316).

Discussion

This is the first time that the effects of long-term placebo-controlled anthelmintic treatment on different components of a helminth-induced Th2 response have been analyzed in a whole community. We show a significant decline in plasma IgE levels in albendazole-treated subjects over a period of 21 months compared to placebo-treated subjects. However, whereas both IgE and IL-5 are important products of the helminth-induced Th2 response, IL-5 production by peripheral blood cells in response to PHA was not significantly affected by anthelmintic treatment and rather tended to increase.

It is well known that helminth parasites are potent inducers of IgE production (12-15). So far, only a few longitudinal studies have been performed to study the effect of anthelmintic treatment on polyclonal IgE levels (16-18). In line with our findings, Cooper et al. showed that one year of anthelmintic treatment was associated with a significant reduction in total IgE levels in children (14) suggesting that current STH infections are important determinants of IgE levels. Whereas previous studies only included children below 12 years of age (14, 16), in the current randomized trial we studied the general population across all ages to show decreases in IgE levels in the whole community irrespective of age.

Overall, the treatment effect on IgE was seen at 9 months and this effect remained at 21 months and IgE levels did not further decrease. . The major effect seen on IgE after 9 months probably reflects the large decrease in prevalence of hookworm and *A. lumbricoides* which was 2.5 and 2.3 fold compared to baseline, respectively, whereas after 21 months the prevalence of both species decreased 1.4 fold compared to 9 months post treatment. The lack of further decrease in IgE at 21 months may be due to continued exposure to incoming parasites in a contaminated environment, which would stimulate long-lived IgE plasma cells contributing to sustained IgE antibody production (14, 19).

Given the observed treatment effect on IgE, it would appear that deworming decreases Th2 mediated responses. However, we found no significant effect of treatment on IL-5 in response to PHA and even a trend towards increase at 21 months post-treatment. As described elsewhere in more detail (10), analysis of uninfected subjects showed that treatment had no effect on IL-5

production, ruling out a direct effect of albenzole on immune responses. It has been reported before that IL-5 responses may be suppressed during helminth infections and treatment results in enhanced helminth-specific and unrelated antigen responses (10, 20-22).

A study conducted in Kenya showed that in schoolchildren infected with *Schistosoma mansoni*, type 2 cytokine responses including IL-4, IL-5, IL-9 and IL-13, were significantly increased upon stimulation with egg antigen 1 and 2 years post treatment (23). Another study in Gabon showed that specifically IL-5, but neither IL-4 nor IL-13, was downregulated during an active *Schistosoma haematobium* infection as only IL-5 responses to worm antigens were increased in subjects who remained free from infection 2 years after helminth clearance, compared to subjects who became reinfected (22).

This enhanced cytokine response can be due to either removal of the immunosuppressive effects of active infection or immunological boosting by antigens released from dying parasites (23). Although it is possible that treatment boosts the antigen specific responses, often also responses to unrelated antigens such as PHA, are enhanced by anthelmintic treatment. The latter cannot be explained by helminth antigens boosting specific T cell responses, unless it is due to cross reactive antigen responses that are enhanced in these unrelated antigens (10, 21).

As anthelmintic treatment appears to have opposite effects on type 2 cytokine production and total IgE, and IL-4 and IL-13 promote IgE production, this raises the question whether there are IL-4/IL-13 independent pathways of IgE regulation. T-cell independent IgE^+-B cell maturation in tissues has been described, suggesting that IgE class switching does not fully depend on Th2 cytokine levels (24). Perhaps IgE memory B cells and/or long-lived IgE plasma cells can maintain the production of IgE via Th2 cytokine-independent pathways, concurrent to Th2 cells becoming hyporesponsive and producing less Th2 cytokines. However, as there is very little data from human studies on IgE memory B cells and the pathways by which IgE is regulated, this remains a speculation.

T follicular helper (Tfh) cells, a subset of $CD4^+$ T cells that migrates to B cell follicles and induces antibody production by B cells (25), have been described as the dominant source of IL-4 *in vivo* during infection with *Heligmosomoides polygyrus* (26). In humans, the frequencies of total and activated peripheral memory Tfh cells were found to be significantly increased during *Schistosoma japonicum* infection (27). Although it is currently unknown what happens to Tfh cell numbers after helminth clearance, decreasing numbers could possibly explain the observed decline in total IgE after treatment, as this would lead to less IL-4 production in B cell follicles, hence less IgE secretion.

Based on our findings, we could conclude that two years of anthelmintic treatment might have differential effects on Th2 responses. IgE responses are elevated in helminth infected subjects and decline after intensive deworming, whereas IL-5 production by peripheral blood cells seems

suppressed in infected subjects and might be reversed after deworming. Both phenomena have separately been reported by others and here we report the occurrence of both within one community. These differential effects on responses typified as Th2 mediated need to be taken into account when examining the effects of helminths on non-communicable diseases. The concern that upon deworming, inflammatory diseases such as allergies could increase, might be justified if we look at Th2 cytokines, but not if one considers IgE. However, it is also known that IgE responses during helminth infection are dominated by antibodies that have poor biological activity (28) and therefore a decrease in IgE that is not functional might still be in line with a relative increase in IgE that can trigger mast cell degranulation.

It would be interesting to complement these findings with data on eosinophilia and other Th2 cytokine responses like IL-4 and IL-13, in order to further investigate helminth's immunomodulatory effects on Th2 responses.

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Authors contribution

KR wrote the paper.

KR and DLT analysed the data.

LW, AEW and FH conducted the field study and performed the experiments.

LvL led the work on PCR detection of parasites.

JWAS supervised the study.

JJH supervised the statistical analysis.

ES coordinated the study.

TS and MY designed and supervised the study.

References

1. http://www.who.int/mediacentre/factsheets/fs366/en/.
2. McSorley HJ and Maizels RM. Helminth infections and host immune regulation. *Clin Microbiol Rev* 2012; **25**: 585-608.
3. Wu D, Molofsky AB, Liang HE *et al.* Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science (New York, NY)* 2011; **332**: 243-247.
4. Hussaarts L, Garcia-Tardon N, van Beek L *et al.* Chronic helminth infection and helminth-derived egg antigens promote adipose tissue M2 macrophages and improve insulin sensitivity in obese mice. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2015; **29**: 3027-3039.
5. Berbudi A, Surendar J, Ajendra J *et al.* Filarial Infection or Antigen Administration Improves Glucose Tolerance in Diet-Induced Obese Mice. *J Innate Immun* 2016; **8**.
6. Yang Z, Grinchuk V, Smith A *et al.* Parasitic nematode-induced modulation of body weight and associated metabolic dysfunction in mouse models of obesity. *Infection and immunity* 2013; **81**: 1905-1914.
7. Wiria AE, Hamid F, Wammes LJ *et al.* The effect of three-monthly albendazole treatment on malarial parasitemia and allergy: a household-based cluster-randomized, double-blind, placebo-controlled trial. *PloS one* 2013; **8**: e57899.
8. Hamid F, Wiria AE, Wammes LJ *et al.* A longitudinal study of allergy and intestinal helminth infections in semi urban and rural areas of Flores, Indonesia (ImmunoSPIN Study). *BMC infectious diseases* 2011; **11**: 83.
9. Wiria AE, Prasetyani MA, Hamid F *et al.* Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). *BMC infectious diseases* 2010; **10**: 77.
10. Wammes LJ, Hamid F, Wiria AE *et al.* Community deworming alleviates geohelminth-induced immune hyporesponsiveness. *Proceedings of the National Academy of Sciences of the United States of America* 2016; **113**: 12526-12531.
11. R-Forge. lme4 - Mixed-effects models project. 2011.
12. Jarrett EE and Miller HR. Production and activities of IgE in helminth infection. *Progress in allergy* 1982; **31**: 178-233.
13. Alcantara-Neves NM, de SGBG, Veiga RV *et al.* Effects of helminth co-infections on atopy, asthma and cytokine production in children living in a poor urban area in Latin America. *BMC research notes* 2014; **7**: 817.
14. Cooper PJ, Alexander N, Moncayo AL *et al.* Environmental determinants of total IgE among school children living in the rural Tropics: importance of geohelminth infections and effect of anthelmintic treatment. *BMC immunology* 2008; **9**: 33.
15. Amarasekera M, Gunawardena NK, de Silva NR, Douglass JA, O'Hehir RE and Weerasinghe A. Impact of helminth infection on childhood allergic diseases in an area in transition from high to low infection burden. *Asia Pacific allergy* 2012; **2**: 122-128.
16. Hagel I, Lynch NR, Di Prisco MC, Rojas E, Perez M and Alvarez N. Ascaris reinfection of slum children: relation with the IgE response. *Clinical and experimental immunology* 1993; **94**: 80-83.
17. Mitre E and Nutman TB. IgE memory: persistence of antigen-specific IgE responses years after treatment of human filarial infections. *The Journal of allergy and clinical immunology* 2006; **117**: 939-945.
18. Poirriez J. A three years follow-up of total serum IgE levels in three patients treated for strongyloidiasis. *Parasite (Paris, France)* 2001; **8**: 359-362.
19. Wu LC and Zarrin AA. The production and regulation of IgE by the immune system. *Nature reviews Immunology* 2014; **14**: 247-259.

20. Cooper PJ, Moncayo AL, Guadalupe I *et al.* Repeated treatments with albendazole enhance Th2 responses to Ascaris Lumbricoides, but not to aeroallergens, in children from rural communities in the Tropics. *The Journal of infectious diseases* 2008; **198**: 1237-1242.
21. Figueiredo CA, Barreto ML, Rodrigues LC *et al.* Chronic intestinal helminth infections are associated with immune hyporesponsiveness and induction of a regulatory network. *Infection and immunity* 2010; **78**: 3160-3167.
22. Grogan JL, Kremsner PG, Deelder AM and Yazdanbakhsh M. Antigen-specific proliferation and interferon-gamma and interleukin-5 production are down-regulated during Schistosoma haematobium infection. *The Journal of infectious diseases* 1998; **177**: 1433-1437.
23. Wilson S, Jones FM, Kenty LC *et al.* Posttreatment changes in cytokines induced by Schistosoma mansoni egg and worm antigens: dissociation of immunity- and morbidity-associated type 2 responses. *The Journal of infectious diseases* 2014; **209**: 1792-1800.
24. Berkowska MA, Heeringa JJ, Hajdarbegovic E *et al.* Human IgE(+) B cells are derived from T cell-dependent and T cell-independent pathways. *The Journal of allergy and clinical immunology* 2014; **134**: 688-697.e686.
25. King C. New insights into the differentiation and function of T follicular helper cells. *Nature reviews Immunology* 2009; **9**: 757-766.
26. King IL and Mohrs M. IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *J Exp Med* 2009; **206**: 1001-1007.
27. Chen X, Li W, Zhang Y *et al.* Distribution of Peripheral Memory T Follicular Helper Cells in Patients with Schistosomiasis Japonica. *PLoS neglected tropical diseases* 2015; **9**: e0004015.
28. Hamid F, Amoah AS, van Ree R and Yazdanbakhsh M. Helminth-induced IgE and protection against allergic disorders. *Curr Top Microbiol Immunol* 2015; **388**: 91-108.

Legend to Figures

Figure 1. Effect of deworming on the prevalence of helminth infections, total IgE levels and whole blood IL-5 production in response to PHA. A) Percentage of helminth infected subjects in placebo and albendazole treated arms. The estimated effect of albendazole treatment on serum total IgE levels (B) and IL-5 response to PHA in supernatant of 72h-stimulated whole blood cultures (C), is displayed for the 9 and the 21 month time points, with corresponding 95% confidence intervals. The estimates of the treatment effect were obtained by general linear mixed models and overall p-values over time are indicated.

Figure S1. Consort diagram. The current study is nested within the ImmunoSPIN trial (8, 9), with a total of 3493 individuals living in the village of Nangapanda. Allocation of placebo and albendazole resulted in 363 and 362 households including 1762 and 1731 subjects, respectively. Subjects were included in the analysis when IgE and/or cytokine data from baseline and at least one of the two follow-up time points were available. While the upper diagram shows the profile with total IgE as outcome, the lower diagram displays the diagram of 250 households that were randomly selected to study immunological responses (IL-5). Availability of parasitological data is indicated at the different time points for treatment arms, numbers reflecting the follow-up of subjects with parasitological data at baseline.

Table 1. Baseline characteristics of the study population

	N	Placebo	N	Albendazole
Age (mean in years, SD)	753	32.2 (18.7)	741	32.4 (19.0)
Sex (female, n, % of total)	753	459 (61.0)	741	445 (60.0)
BMI > 19 years old (mean, SD)	490	22.5 (4.0)	486	21.9 (3.7)
Z score of BMI ≤ 19 years old (mean, SD)	254	-1.17 (1.17)	235	-1.27 (1.18)
Parasite infection (n, %)				
Helminth (any *spp*)	421	357 (84.8)	426	361 (84.7)
Hookworm[1]	439	315 (71.8)	438	326 (74.4)
N. americanus	439	310 (70.6)	438	324 (74.0)
A. duodenale	439	27 (6.2)	438	23 (5.3)
A. lumbricoides[1]	439	154 (35.1)	438	146 (33.3)
T. trichiura[2]	558	144 (25.8)	541	150 (20.2)
Total IgE (IU/ml [GM, 95 CI])	753	1372 (1239-1519)	741	1340 (1210-1485)
Interleukin-5 production in response to PHA (pg/ml [median, IQR])	374	511 (287-777)	308	490 (291-808)

[1] *diagnosed by PCR,* [2] *diagnosed by microscopy*