CHAPTER 6

Three-monthly albendazole treatment alleviates geohelminth-induced immune hyporesponsiveness; results of a double blind placebo-controlled household-randomized trial

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Abstract

Background Chronic helminth infections are proposed to induce cellular immune hyporesponsiveness, which secures their long-term survival in their host, but which also may affect immune responses to unrelated antigens. As there are several other causes of immunosuppression, we conducted a household-clustered RCT to evaluate the specific effect of deworming on cellular immune responses in a helminth-endemic area in Indonesia.

Methods Cytokine (IL-2, IL-5, IL-10, IFN-γ and TNF) responses to antigens and mitogens were assessed in 1059 subjects at baseline, 9 and 21 months after three-monthly treatment with either albendazole or placebo.

Results This intensive treatment resulted in significant increase in malaria-specific and mitogen-induced TNF and IFN-γ responses. This effect was not associated with changes in cell counts or BMI.

Conclusions These findings establish unequivocally that helminth infections suppress pro-inflammatory responses, which may help to understand the possible protective effect of helminths on inflammatory diseases in rural areas of the world.

Introduction

Infection with soil-transmitted helminths (STH) is the most common infectious disease worldwide and affects mostly inhabitants of rural areas in low- and middle-income countries[1]. In addition to causing direct worm-associated morbidities, chronic STH infections may magnify poor health conditions common in communities remote from health care facilities, such as anemia, poor nutritional status, stunting and possibly poor cognitive development[1,2].

An important hallmark of chronic helminth infections is cellular hyporesponsiveness, which is thought to allow the long-term survival of these parasites within their host[3,4]. Although unresponsiveness in lymphocyte proliferation was already described in the 1970s for individuals with *Schistosoma mansoni* infection or bancroftian filariasis[5,6], the evidence for this has not moved beyond animal models and cross-sectional studies in humans (reviewed by Danilowicz-Luebert et al.[7]). An important drawback to the cross-sectional nature of these studies is that other factors, which are also associated with immune suppression, could bias the results. Individuals infected with helminths may be in a poor nutritional state and shortage of proteins or amino acids can interfere with expression of immune effector molecules. Malnutrition has been specifically associated with decreased cell-mediated immunity, exemplified by atrophy of the thymus and other lymphoid tissues leading to lower T-cell numbers and reactivity[8]. It is also known that other microorganisms and parasites can be associated with immune suppression or T-cell exhaustion[9] and therefore coinfections could act as confounders[10].

The consequences of immunosuppression are manifold and could be of major public health importance. Immune hyporesponsiveness, in the presence of helminth infections, can affect responses to unrelated antigens, it could curtail the development of effective immune responses to incoming protozoan, bacterial or viral infections, thereby increasing susceptibility to these pathogens. Similarly, vaccination studies have shown suboptimal responses to childhood vaccinations in subjects infected with STH[11,12]. On the other hand, the dampened immune responses associated with helminths might help to prevent immune-induced pathology during coinfections and, possibly, overt reactivity to self- or environmental antigens[13].

A few longitudinal studies have been undertaken to assess the effect of anthelmintic treatment on cellular immune responsiveness, however these were in small number of subjects or specifically targeted children[14-16]. Conversely, clinical trials that have experimentally infected humans have mostly not evaluated cellular immune responses and have all been conducted in adults[17,18]. Moreover, therapeutic infections are often not long enough to establish a chronic infection, which could be important for the gradual onset of hyporesponsiveness. So far

there are no large-scale community-based intervention studies that show helminth infections lead to immune hyporesponsiveness in man.
To disentangle the impact of helminths on the immune system from other influences, we conducted a randomized double blind placebo-controlled trial of three-monthly single dose albendazole treatment in an area where STH are highly endemic. Here we present the results of our trial; the effect of anthelmintic treatment on the peripheral blood cytokine responses of a community in Flores island, Indonesia.

Methods

Study design

This report describes a nested study within the ImmunoSPIN trial[19,20]. The trial was conducted in two villages in Ende district, Flores island, Indonesia. The coastal village Nangapanda is located around the main road of Flores and can be characterized as semi-urban, based on the location and the presence of a primary healthcare centre. Anaranda village is located 80 km north of Nangapanda and is more remote from roads, health centres and other facilities. In 2008 the double blind placebo-controlled trial of two year duration was initiated by randomizing all households in the two villages 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 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 (according to Indonesian national guidelines), and 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 in the two villages, comprising of 4004 individuals, resulting in 2022 (481 houses) and 1982 (473 houses) subjects in placebo and albendazole group, respectively. For the immunological component of this study in Nangapanda, aiming at 1000 participants, 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 from the semi-urban area, of which 858 provided sufficient blood samples for whole blood cultures. In the rural area Anaranda, only children were included since this area was included for our allergy studies[19]. 250 children were randomly selected from the total population and children from the same households were also included, leading to a total number of 295 children with whole blood cultures. After exclusion of cytokine data from wells that were suspected of being infected (see below), the number of subjects included at baseline was 839 and 220 for the two respective areas, corresponding to 572 placebo- and 487 albendazole-treated individuals.

Whole blood culture and cytokine measurements

Heparinized blood was diluted 1:4 with RPMI 1640 medium (Invitrogen, Breda, the Netherlands) and cultured in 96-well round-bottomed plates. Cultures were stimulated for 24h to assess innate responses (to lipopolysaccharide (LPS) from *E. coli*, Sigma-Aldrich, Zwijndrecht, the Netherlands), and for 72h to detect adaptive responses (to *Ascaris lumbricoides* antigen, *Plasmodium falciparum*-parasitized red blood cells (PfRBC), uninfected RBC (uRBC) and phytohaemagglutinin (PHA, Wellcome Diagnostics, Darford, UK)) and at each time point unstimulated control wells were included. PfRBC and uRBC were kindly provided by professor Sauerwein from Radboud University Medical Center Nijmegen, the Netherlands and were only used in the semi-urban area, since the rural area was not endemic for malaria. The cultures were carried out in the field laboratory in Nangapanda and the supernatants were kept at -20°C and transported to Jakarta. There cytokine responses were quantified using Luminex cytokine kits (Biosource, Camarillo, CA, USA) and run on a Liquichip 200® Workstation (Qiagen, Venlo, The Netherlands) equipped with Liquichip analyzer software (Qiagen, Venlo, The Netherlands). TNF and IL-10 were assessed in 24h supernatants and TNF, IFN-γ, IL-2, IL-5 and IL-10 in 72h supernatants. Samples with TNF levels ≥250 pg/mL in unstimulated blood were excluded from the analyses as they are considered unreliable with respect to possible infection in the culture. This value was derived from the data distribution, which indicated outliers to be identified with this cut-off. Cytokine levels that fell below the assay's detectable range were replaced by half of the detection limit.

Stool examination by microscopy and PCR

In order to examine the effect of treatment on helminth prevalence, yearly stool samples were collected. *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 (*Ancylostoma duodenale*, *Necator americanus)*, *A. lumbricoides*, and *Strongyloides stercoralis* DNA, as described previously[20].

Complete blood counts

Complete blood counts (CBC) and differential counts before and one year after treatment were determined using heparinized blood on a routine cell counter (Coulter® Ac-T™ diff Analyzer, Beckman Coulter Inc., Fullerton, CA, USA), while CBC 2 years after treatment were determined using heparinized and EDTA blood on Sysmex KX-21N hematology analyzer (PT Sysmex Indonesia, Jakarta, Indonesia). Since both heparinized and EDTA blood samples were used at the last time point, 325 samples were tested in parallel analysis. All outcomes were highly

comparable except for thrombocyte counts, thus the data of all parameters but thrombocyte counts were pooled.

Statistical analysis

The cytokine data were log transformed (log10(concentration+1)) to obtain normally distributed variables. For children ≤19 years, BMI age-standardized z-scores were calculated according to WHO references[21]. 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. Linear or logistic mixed-effects models[22] were applied for continuous and binary outcomes, respectively. Parameter estimates for treatment effects at 9 and 21 months and 95% confidence intervals are reported. The reported p-values are obtained using likelihood ratio tests by comparing the model with and without the treatment effect. Unless stated otherwise, all outcomes were adjusted for area by using area as covariate in the model. All models were fitted using the lme4 package[23].

Results

Study population

The baseline characteristics of the study participants are shown in table 1. At baseline 88.7% of the individuals were infected with one or more helminth species, with hookworm infection being the most prevalent (77.1% of total). The consort diagram of the trial is shown in figure 1; follow-up after 9 months was 88% for both groups and 76% for placebo- and 75% for albendazole-treated groups after 21 months, corresponding to a total loss of 138 and 123 individuals respectively. Six subjects died during the study period, which were all above the age of 35, suggesting non-infectious causes of death. The analysis was intention-to-treat, and involved all participants as assigned randomly at the start of the trial. No significant change in BMI was observed over the 2-year study period in children (analyzed by zBMI, p=0.70) or in adults (BMI, p=0.45; data not shown).

Effect of albendazole treatment on helminth prevalence

Treatment with albendazole resulted in a reduction in the prevalence of geohelminths both after 9 (51.9% vs. 84.1% for placebo) and after 21 months (39.2% vs. 80% for placebo) (Table S1). Albendazole had the most effect on hookworm (from 78.4% at baseline to 32.6% at 9 months and 21.6% at 21 months post-treatment) compared to placebo (from 76.1% to 70.5% and 67.0% respectively), followed by on *Ascaris* (albendazole from 32.7%, to 13.3% and 12.2%; placebo from 31.3% to 33.5% and 24.2%), while the effect on *Trichuris* was less pronounced (from 20.0% at baseline to 21.0% at 9 months and 16.2% at 21 months post-treatment, compared to placebo from 25.5% to 31.7% and 28.8%). When assessing the intensity of infection in categories based on cycle threshold values of PCR, it was in particular the high-load infections that were greatly diminished in the treatment group[24].

Table 1. Baseline characteristics of the study population

	N	Placebo	N	Albendazole
Age (mean in years, SD)	572	25.7 (18.5)	487	24.9 (18.4)
Sex (female, n, % of total)	572	328 (57.3)	487	279 (57.3)
Area (rural, n, % of total)	572	114 (19.9)	487	106 (21.8)
BMI > 19 years old (mean, SD)	264	22.1 (4.1)	220	22.1 (3.8)
Z score of BMI ≤ 19 years old (mean, SD)	194	-1.15 (1.11)	386	-1.14(1.15)
Parasite infection (n, %)*				
Helminth *(*any *spp)*	322	286 (88.8)	237	210 (88.6)
- Hookworm[1]	335	255 (76.1)	245	192 (78.4)
N. americanus[1]	335	252 (75.2)	245	188 (76.7)
A. duodenale[1]	335	25 (7.5)	245	17 (6.9)
- *A. lumbricoides*[1]	335	105 (31.3)	245	80 (32.7)
- *S. stercoralis*[1]	335	3 (0.9)	245	14 (5.7)
- *T. trichiura*[2]	415	106 (25.5)	310	62 (20.0)
Malarial parasitemia *(*any *spp)*[2]	567	24 (4.2)	483	24 (5.0)
- *P. falciparum*	567	16 (2.8)	483	11 (2.3)
- *P. vivax*	567	8 (1.4)	483	10 (2.1)
- *P. malariae*	567	0 (0.0)	483	4 (0.8)
Cytokine production (pg/mL [median, IQR])				
LPS				
TNF	554	743 [368-1293]	468	769 [339-1318]
IL-10	554	271 [163-441]	468	256 [158-406]
PHA				
TNF	516	100 [50-222]	435	103 [50-214]
IL-10	515	76 [41-129]	435	70 [37-116]
IFN-γ	516	1625 [584-3983]	435	1270 [538-4340]
IL-2	516	23 [0-101]	432	23 [0-92]
IL-5	516	563 [309-840]	435	520 [317-829]
PfRBC				
TNF	299	18 [4-42]	237	14 [3-38]
IL-10	300	10 [5-19]	238	10 [5-20]
IFN-γ	300	163 [75-388]	239	176 [70-376]
IL-2	300	50 [5-125]	239	40 [5-112]
IL-5	300	14 [5-26]	239	12 [4-23]
Ascaris				
TNF	517	5 [0-15]	438	6 [0-14]
IL-10	516	7 [2-15]	438	7 [1-14]
IFN-γ	516	19 [6-47]	441	21 [7-47]
IL-2	497	38 [4-114]	426	36 [0-107]
IL-5	515	24 [9-68]	440	24 [9-63]

[1]diagnosed by PCR; [2]diagnosed by microscopy.
**The number of positives (n) of the total population examined (N)*

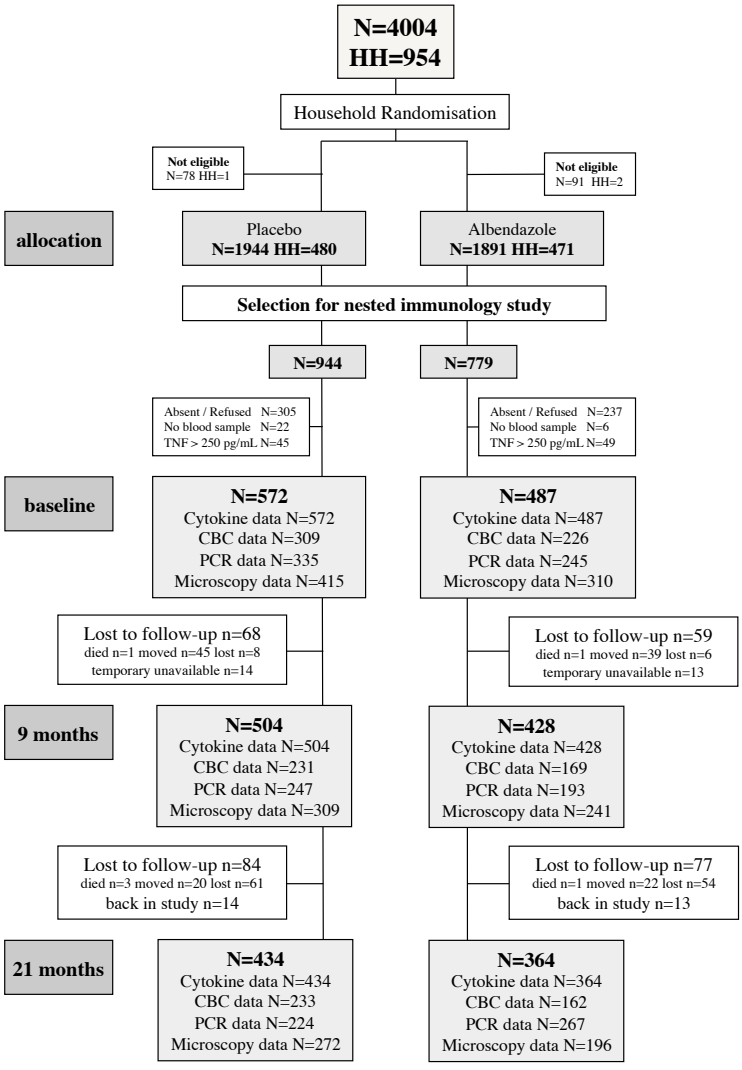

Figure 1. Consort diagram. The current study is nested within the ImmunoSPIN trial, with a total of 4004 individuals in two participating villages. Allocation of placebo and albendazole treatment resulted in 480 and 471 households including 1944 and 1891 in the two groups, respectively. For the immunological studies, a random selection was made and 1723 individuals were invited to participate (n=944 and n=779 respectively). Cytokines were assessed for 1059 subjects, of which 572 in the placebo and 487 in the albendazole arm. After 9 months 504 and 428 and after 21 months 434 and 364 individuals were analyzed, in placebo and albendazole group, respectively. Availability of complete blood counts (CBC) and parasitological data is indicated at the different time points for both groups.

Effect of albendazole treatment on whole blood cytokine responses

In figure 2, we present the effect of treatment on cytokine responses to *Ascaris* antigens, PfRBC and PHA. The model-estimated treatment effects on cytokines at 9 months (figure 2A) and 21 months (figure 2B) are shown. Regarding helminth (*Ascaris*) antigen-specific cytokine responses, IL-2 responses were significantly enhanced by treatment over the study period (p_{time}=0.018), with significantly higher IL2 in the treated group at 9 months (estimate [95% CI]: 0.17 [0.05–0.28], figure 2A). Neither Th1, nor Th2 responses changed with treatment. In response to *P. falciparum* antigens, there was an increase over time in pro inflammatory cytokine TNF, which was highly significant (p_{time}<0.0001), and IFN-γ (p_{time}=0.036), in the albendazole-treated group. As shown in figure 2A, both TNF and IFN-γ were significantly higher than in the placebo treated group at the 9 months time point (0.37 [0.21-0.53] for TNF and 0.14 [0.03-0.24] for IFN-γ). Moreover, the general adaptive response (cytokine production stimulated by PHA), albendazole treatment significantly increased TNF and IL-10 secretion (p_{time}=0.011 and p_{time}=0.003 respectively) over the trial period. Interestingly, for TNF, albendazole treatment resulted in elevated response at 9 months whereas for IL-10 the response was significantly higher at the later 21 months time point (for TNF 0.14 [0.05–0.24], figure 2A; for IL-10 0.12 [0.05–0.19], figure 2B). At 9 months post-treatment, IFN-γ (0.10 [0.01-0.19]) and IL-2 (0.12 [0.01-0.23]) responses were transiently increased (figure 2A) and at 21 months a significant enhancement of IL-5 production (0.10 [0.01-0.19]) was observed (figure 2B), however these alterations were not significant over the whole trial time period (IFN-γ p_{time}=0.076, IL-2 p_{time}=0.11, IL-5 p_{time}=0.068). Albendazole had no effect on immune responses to LPS (overall p-value for TNF p=0.77, for IL-10 p=0.12, data not shown). Analysis of cytokines in unstimulated whole blood cultures revealed no treatment-related differences (data not shown). When assessing responses to uRBC as control for PfRBC, we found that IFN-γ levels were not different between the treatment arms (p=0.91), however TNF production was increased post-treatment in the albendazole arm, although to a lesser extent than what was seen in response to PfRBC (p=0.018; figure S1).

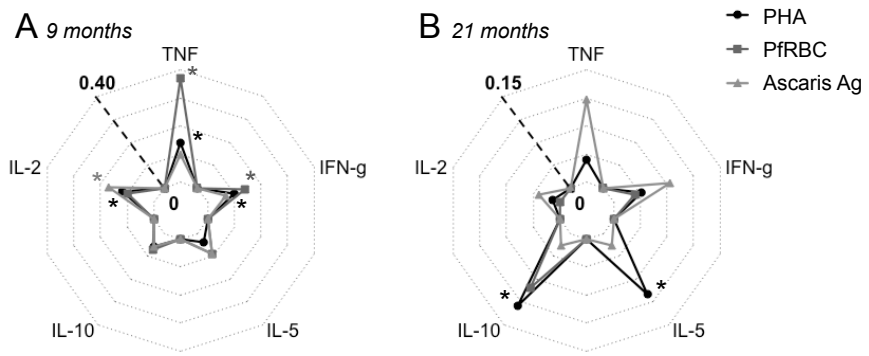

Figure 2. Effect of deworming on cytokine responses to *Ascaris*, PfRBC and PHA. TNF, IFN-γ, IL-2, IL-5 and IL-10 concentrations were assessed in supernatants of 72h-stimulated whole blood cultures. The effect of albendazole treatment on cytokine responses to PHA (black circles), PfRBC (dark grey squares) and *Ascaris* (light grey triangles) is shown. The estimates of the treatment effect in the whole study population after 9 (A) and 21 (B) months of albendazole treatment were obtained by general linear mixed models; asterisks with corresponding colors (black for PHA, dark grey for PfRBC, light grey for *Ascaris*) indicate a significant effect.

Increase in pro-inflammatory responses after treatment in helminth-infected individuals

To determine whether the enhanced cytokine responses could be due to a direct effect of albendazole, we stratified the analysis based on STH infection status at baseline. The enhancement of mitogen- as well as malaria-induced TNF by albendazole treatment was observed in helminth-infected individuals (overall p-values for PHA and PfRBC were p_{time}=0.098 and p_{time}=0.0004 respectively, figure 3A and 3B), but not in uninfected ones (data not shown). Importantly, uRBC-induced TNF responses were not increased in either helminth-infected or uninfected subjects (data not shown). Also in the response to *Ascaris* antigen, enhancement of TNF was observed in the stratified analysis of helminth-positives at baseline (p_{time}=0.044, figure 3C) but not in helminth negatives (data not shown). Moreover, elevated IFN-γ and IL-2 responses to Ascaris were only observed after treatment of the helminth-infected (p_{time}=0.028 and p_{time}=0.006 respectively; not shown).

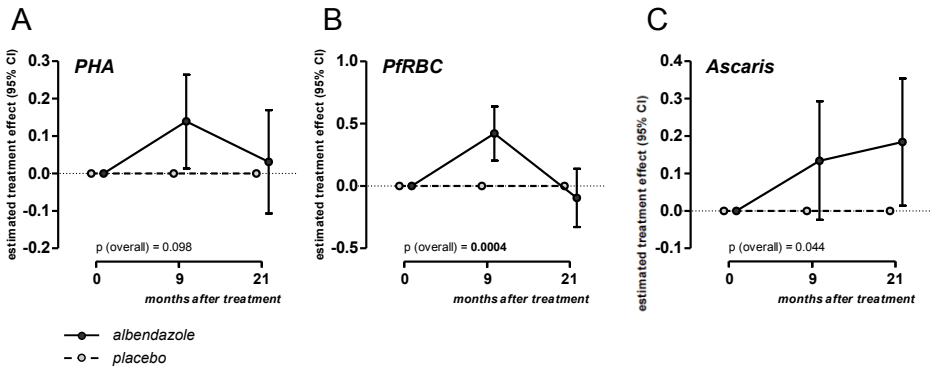

Figure 3. Effect of deworming on TNF responses in helminth-infected. TNF secretion was measured in supernatants from whole blood stimulated with (A) PHA, (B) PfRBC and (C) *Ascaris* antigen for 72 hours. The estimated effect of albendazole treatment on TNF responses in helminth-infected subjects is displayed for the 9 and 21 months time points, with corresponding 95% confidence intervals. The estimates of the treatment effect were obtained by general linear mixed model and overall p-values over time are indicated.

Increased cytokine responses are not associated with changes in cell counts

The total leukocyte count increased in the albendazole group at 9 months post-treatment and was similar in both groups at 21 months (p=0.035 and p=0.14 respectively; data not shown). However, we observed a negative association of leukocyte counts and both TNF and IFN-γ responses to PfRBC, indicating that increased leukocyte numbers could not be responsible for the enhanced cytokine responses after treatment. When analyzing differential cell counts and proportions, the lymphocytes and granulocytes did not change after treatment, whereas monocyte proportions and numbers were higher in the albendazole group. No association was found between monocyte numbers and cytokine production in response to any of the stimuli (data not shown). No treatment effect was noted on thrombocyte or erythrocyte counts, hemoglobin levels or hematocrit.

Discussion

This is the first time that helminth-specific and -unrelated cytokine responses have been analyzed in a whole community before and after repeated long-term placebo-controlled anthelmintic treatment. We show that treatment of STH infections increases cytokine responses, with profound effects on helminth-specific and other adaptive immune responses, providing conclusive evidence for helminth-induced immune hyporesponsiveness in humans.

Most pronounced were elevated pro-inflammatory, TNF and IFN-γ, cytokine responses after stimulation with mitogen and malarial antigens. Albendazole is a drug, which might induce production of inflammatory cytokines in a human monocytic cell line[25]. Stratifying the analysis for helminth infection status at baseline revealed stronger effects in the helminth-infected group, indicating that the suppression of pro-inflammatory cytokine responses is unlikely to be due to direct effects of albendazole, but can be regarded as a true helminth-induced phenomenon.

Subsequent to the rise in pro-inflammatory responses at 9 months, an interesting finding was the enhancement of IL-5 and IL-10 responses at 21 months post-treatment. Although helminth infections skew the immune system towards type 2 responses, suppression of these responses during helminth infections has been reported before in studies comparing helminth infected and uninfected subjects[26,27]. IL-10 upregulation appears particularly surprising; as it has been postulated that helminth-associated inhibition of pro-inflammatory responses is mediated by this suppressory cytokine[28]. Increased IL-10 responses after anthelmintic treatment have previously been observed in schistosomiasis[29] and STH infection[16]. Whether the increased pro-inflammatory responses in the first year leads to higher IL-10 in the second year to prevent overt inflammation, is not clear from these data. Moreover, it is known that IL-10 can be part of the Th2 response and therefore the increased IL-10 might be a component of the enhanced Th2 response following deworming, leaving the question whether IL-10 originating from other cells is involved in cellular hyporesponsiveness caused by helminth infections. However, the fact that different cytokines appear to all increase in response to antigens and mitogens after anthelmintic treatment seems to indicate that all adaptive immune responses are enhanced after deworming. This would predict that there is a general helminth-mediated hyporesponsiveness which is neither restricted to a particular pro- or anti-inflammatory nor to a Th1 or Th2 response, but might stem from a common general effect such as alternation in cell counts, changes in nutrients essential to functioning of the immune system or suppressory cells and factors which do not involve IL-10.

Importantly, cell counts were affected by reduction in helminths, but did not show any correlation with cytokine responses, excluding the possibility that the general

increase in responsiveness is due to higher numbers of cytokine-secreting cells. As immune responses can be enhanced by improved energy resources, we assessed BMI, and fasting glucose level (not shown), as proxies for nutritional status but these parameters were not affected by deworming.

The three-monthly albendazole treatment over a two-year period was not effective in eliminating all helminth infections. Treatment efficacy was particularly low for *Trichuris*, shown in earlier studies that used single or double doses of albendazole and / or mebendazole[30,31]. Here we show that even 7 doses of albendazole over a 21 month period is not sufficiently effective against *Trichuris* infection. By using a household-clustered design for randomization, repeated treatments and observed intake, we had expected a more effective reduction in transmission of STH. For better deworming results, more intensive treatment or inclusion of environmental control would be needed. However, it is clear that even a reduction in helminth infections in the community can lead to alleviation of immune hyporesponsiveness and that a more effective deworming, might result in even more pronounced immunological effects.

There is an increasing awareness that helminths might play an essential role in the development and homeostasis of the human immune system[32,33]. In areas where chronic helminth infections are persistent, the immune system may have evolved to operate optimally in the face of helminth-induced downmodulation; any disturbance of the long evolutionary coexistence between humans and helminths might be associated with the emergence of pathological conditions[34]. The question of what the clinical consequences of the enhanced adaptive immune responses are following deworming will need to be addressed next. So far, our trial of three-monthly albendazole treatment over a 21-month period did not show clear clinical changes[24]. Although we found a transient increase in malarial parasitemia at 6 months post treatment, the time point after the rainy season, this could not be confirmed during the further follow-up period, as the prevalence of malaria decreased drastically in the study area. With respect to allergy, skin prick test (SPT) reactivity was assessed in school children in our study cohort. This revealed a specific increase in SPT reactivity to cockroach allergens after two years of anthelmintic treatment, but no effect on allergy symptoms[24]. The effects of anthelmintic treatment on other infectious or inflammatory diseases should probably be investigated over a longer period, since the immunomodulatory effects of helminths are likely to have developed over several years of chronic infection. In the case of allergic diseases this is illustrated by a recent publication, showing an increase in allergen SPT reactivity and possibly eczema symptoms after more than 15 years of ivermectin treatment[35], whereas studies with a shorter treatment course failed to show increased SPT reactivity or symptoms[14,15].

Several clinical trials are underway to evaluate the possible beneficial effect of helminth infection or their excretory products on the symptoms and prevalence of

inflammatory diseases[36,37], while at the same time efforts are made to control STH by implementing regular treatment programs in developing countries[38]. These studies, if conducted within appropriate time frames, should be able to answer the question what clinical consequences of human helminth infections are. Given our results that there are major effects on immune responses following deworming, it will be important to include immunological measurements in future deworming programs in order to understand causation and predict clinical outcomes.

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Supplementary material

Table S1. Prevalence of helminth infections at post-treatment time points.

		N	any spp	*Hookworm*[1]	*A. lumbricoides*[1]	*T.trichiuria*[2]
			n (%)	n (%)	n (%)	n (%)
9 months	Placebo	227	191 (84.1%)	160 (70.5%)	76 (33.5%)	72 (31.7%)
	Albendazole	181	94 (51.9%)	59 (32.6%)	24 (13.3%)	38 (21.0%)
21 months	Placebo	215	171 (80.0%)	144 (67.0%)	52 (24.2%)	62 (28.8%)
	Albendazole	148	58 (39.2%)	32 (21.6%)	18 (12.2%)	24 (16.2%)

[1]diagnosed by PCR. [2]diagnosed by microscopy.

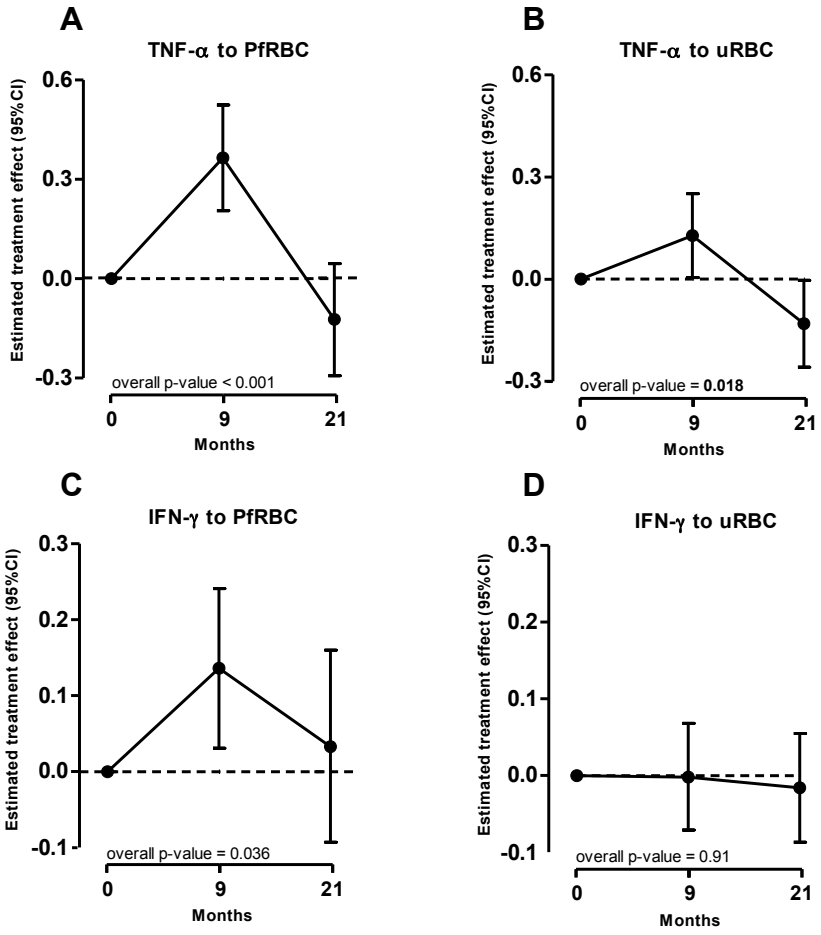

Figure S1. Effect of deworming on TNF and IFN-γ responses to PfRBC and uRBC. TNF (A, B) and IFN-γ (C, D) responses were measured after 72h of stimulation with *Plasmodium falciparum*-infected and -uninfected RBC (PfRBC (A, C) and uRBC (B, D), respectively). The estimated effect of albendazole treatment on TNF responses in helminth-infected subjects is displayed for the 9 and 21 months time points, with corresponding 95% confidence intervals. The estimates of the treatment effect were obtained by general linear mixed model and overall p-values over time are indicated.