Association of deworming with reduced eosinophilia: implications for HIV/AIDS and co-endemic diseases

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Eosinophil counts in venous blood were monitored during a randomized controlled deworming trial (n = 155 children) that lasted for a year, and in a whole-school deworming programme (range 174–256 children) of 2 years' duration. Mean eosinophil counts ($\times 10^9$/l) decreased from 0.70 in the randomized trial, and 0.61 in the whole-school study, to well within the normal paediatric range of 0.05–0.45 ($P < 0.05$). The prevalence of eosinophilia declined from 57% to 37% in the randomized trial (mean for 400, 800 and 1200 mg albendazole doses); and from 47% to 24% in the whole-school study (500 mg stat mebendazole). Benzimidazole anthelminthics were highly effective against *Ascaris* but less so against *Trichuris.* Activated eosinophils are effector and immunoregulatory leucocytes of the T-helper cell type 2 (Th2) immune response to parasitic helminths and atopic disorders. Under conditions of poverty where soil-transmitted helminths are hyperendemic, Th2 polarization of the immune profile is characteristic. Regular anthelminthic treatment should reduce contact with worm antigens, and this may contribute to re-balancing of the immune profile. Suppression of eosinophil recruitment and activation, together with related cellular and molecular immunological changes, might have positive implications for prevention and treatment of co-endemic diseases, including HIV/AIDS, cholera, tuberculosis and atopic disorders.

Eosinophilia is indicative of a humoral immune response. Under conditions of poverty, this kind of profile is often the result of sustained contact with helminthic antigens; whereas antigens that give rise to atopy may be the main cause under conditions of affluence. Infestation of humans by various parasitic helminths is widespread in South Africa.[1–4] The epidemiological and public health importance of helminth-induced eosinophilia includes the possibility that underlying chronic immune activation creates the potential for interaction with co-endemic diseases and preventive vaccination.[5–26] When pregnant women are parasitized by worms in Africa, and probably elsewhere as well, even the foetus becomes sensitized to helminths, and the T-cell priming persists into childhood.[23] This perinatal T-cell imbalance could influence susceptibility to infection by intracellular pathogens, efficacy of vaccines, and development of immune-mediated disorders.[5–26] The purpose of this paper is to describe deworming interventions at two South African schools, during which significant reductions in mean eosinophil counts, and reductions in the prevalence of eosinophilia, were sustained; and to consider briefly the implications in the context of co-endemic human helminthiasis, other infections, and allergies.

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Methods

The effects on eosinophilia of the anthelminthics, albendazole and mebendazole, were determined during a randomized controlled trial and a whole-school deworming programme. Free and informed consent to treat the children with anthelminthic tablets as the only medication, and to draw blood from them, was obtained from all parents or guardians, and from the school committees. Both of the projects were approved by the Ethics Committee of the South African Medical Research Council.

Randomized controlled trial

The original objective was to benchmark the efficacy of albendazole (Zentel® 400 mg tablets, SmithKline Beecham) against the whipworm *Trichuris trichiura* in the Mediterranean-type climate (winter rainfall area) of the Western Cape Province of South Africa, in terms of cure rate and egg reduction rate.[27] The participants (n = 155) were children of wine-farm labourers, attending a primary school in the Boland region,100 km east of Cape Town. Median age at commencement was 112 months (range 78–174 months). Albendazole doses of 400, 800 or 1200 mg were each administered four times at intervals of approximately four months. The main inclusion criterion for treatment with albendazole was the presence of *Trichuris* eggs in a faecal sample, resulting in a prevalence of 100% in three groups matched for age, gender and the number of *Trichuris* eggs per gram of stool. Allocation of a group of children to a particular dose of albendazole was by a randomized process. Concomitant infection with the common roundworm *Ascaris lumbricoides* occurred in 47%, 50%, 54% and 0% of children who received 400, 800 or 1200 mg of albendazole, or placebo, respectively.[27] There was an ethical directive that children known to have worms must not be treated with placebo. Therefore, individuals with no eggs in their faeces when the study commenced were dosed with placebo tablets and served as a negative control (or reference) group. Appearance and flavour of albendazole and placebo tablets matched exactly, and each tablet was blister-packed in France. Sets of three packs of albendazole and/or placebo for treatments spanning three days at 400 mg/day, were prepared in the laboratory. At the school, administration of each set to the corresponding child was double blind. Before deworming started and after two and four deworming treatments, blood was drawn from the antecubital vein into vacuum tubes containing EDTA. Differential white blood cell counts were obtained by means of a Technicon H2 blood cell analyser (Technicon, Tarrytown, New York). Counts that exceeded normal ranges were checked by microscopy. The laboratory reported results in relation to the date of birth, but had no other specific knowledge of the case. Repeated-measures analysis of variance was the statistical method used to test eosinophil counts for dose effects, and for interactions with time, age and gender. Counts were made in all seasons of the year.[27]

Whole-school deworming programme

A community on the west coast, 135 km north of Cape Town, requested assistance with implementation of a deworming programme in their primary school.[1] Intervention was recommended after it had been shown that 72% of children were infected by *Trichuris* and 19% with *Ascaris.* The schedule was to deworm all the children at intervals of approximately four months with a dose of one 500 mg mebendazole tablet *per os*

Table 1. Changes in mean eosinophil counts and prevalence of eosinophilia in children: randomized controlled trial (worm prevalence before and after deworming is summarized in the first footnote).

Treatment and number of children	Before deworming		After 2 dewormings[a]		After 4 dewormings[a]	
	Means[a] (s.e.m.)[b]	% > 0.45[c]	Means (s.e.m.)[b]	% > 0.45[c]	Means (s.e.m.)[b]	% > 0.45[c]
400 mg[d] (n = 37)	0.676 (0.087)	51	0.382 (0.035)	35	0.397 (0.037)	35
800 mg[d] n = 41)	0.762 (0.080)	66	0.548 (0.063)	49	0.426 (0.038)	41
1200 mg[d] (n = 40)	0.643 (0.075)	50	0.401 (0.071)	33	0.459 (0.061)	38
Reference[e] (n = 37)	0.384 (0.037)	29	0.342 (0.031)	27	0.472 (0.046)	49

Worm prevalence before and following deworming with albendazole: *Trichuris* 100% before and afterwards ranged from 33.3% for the 1200-mg dose to 77.4% for the 400-mg dose; *Ascaris* 50.3% before and 0% afterwards.

[a]After two and four dewormings, eosinophil counts were significantly reduced for all the doses of albendazole, with respective P-values of <0.01 and <0.05.

[b]S.e.m., standard error of the mean.

[c]Prevalence of eosinophilia. The percentage of children with eosinophil counts exceeding the normal paediatric range for eosinophils in venous blood: $0.05 - 0.45 \times 10^9$/l.[28,29] The mean prevalence in the combined albendazole groups decreased from 57% before to 37% after deworming.

[d]Albendazole *per os,* as Zentel® 400 mg tablets, SmithKline Beecham: treatment intervals approximately four months, compliance observed.

[e]Children in this negative control group were treated with placebo only. They had no worms when the study began, but infections developed progressively, which probably explains the increases in eosinophil counts and prevalence of eosinophilia.

(Vermox®, Janssen-Cilag). Median age at commencement was 111 months (range 46–218 months). Children were dewormed six times. The number of children available for venipuncture on different occasions ranged from 174–256, depending on absenteeism and voluntary participation. Blood cells were counted in the same laboratory and by the same methods as for the randomized controlled trial. Differential white blood cell counts defining eosinophilia were done before and after two, four and six dewormings, respectively, utilizing all children who presented for venipuncture. Changes in eosinophil counts were evaluated statistically by repeated-measures analysis of variance. Counts were made in all seasons of the year.

Results

The normal paediatric range for eosinophil counts at the tertiary hospital (Tygerberg Hospital) serving both the study populations is $0.05–0.45 \times 10^9$/l.[28,29]

Randomized controlled trial

Children who left the school, reduced numbers from the original allocation of 25 boys and 25 girls per treatment (Table 1).[27] Compliance by ingestion of anthelminthic tablets was 100% in pupils who completed the study. The mean eosinophil count exceeded the upper limit of the normal paediatric range (0.45×10^9/l)[28,29] before treatment with albendazole began (Table 1). Sustained reductions in mean eosinophil counts were statistically significant after two and four dewormings, and were accompanied by reductions in the prevalence of eosinophilia, for all the doses of albendazole. There was no clear dose-related response but the 800 mg treatment was associated with the largest reduction in counts (Table 1). There was a trend for higher counts to decrease more in all the groups treated with albendazole, in which prevalence of helminthiasis was originally 100%. In these groups, but not in the placebo group, the mean counts for successive ascending quartiles declined from before treatment by 0%, 6%, 40% and 57% per quartile, after four treatments. Eosinophilia was associated with helminthiasis *per se,* rather than differentially with the severity of either trichuriasis or ascariasis, or with concomitant infections by both helminths (data not shown). There were no significant interactions with gender, age or time. By the end of the study, incidences per annum of 15% trichuriasis and 29% ascariasis in the originally uninfected negative control group (treated with placebo), were associated with increased mean eosinophil counts and the prevalence of eosinophilia. Other leucocyte counts were not influenced by the treatments. All the doses of albendazole were highly effective, according to international criteria,[30] against *Ascaris* in terms of cure rates and egg reduction rates; whereas only the 800 and 1200 mg doses were effective against *Trichuris* (data not shown).

Whole-school deworming programme

Before deworming, the mean eosinophil count for the whole school exceeded the upper limit of the normal paediatric range, namely, 0.45×10^9/l (Table 2).[28,29] Treatment compliance ranged from 73% to 100% (mean 91.2%) and results are summarized in Table 2. During the deworming programme, there were sustained reductions in mean eosinophil counts and the prevalence of eosinophilia. Decreases in counts were significant after two and four dewormings, and approached significance between the fourth and sixth treatments. Despite the constraints of study design, there was again an implied trend for higher counts to respond more to deworming. This was shown by reduction in the mean counts for successive ascending quartiles from before treatment, by 0%, 24%, 44% and 56% per quartile, after six treatments. Again, eosinophilia was associated with helminthiasis *per se,* rather than differentially with the intensity of either trichuriasis or ascariasis, or with concomitant infections by both helminths (data not shown). Other leucocyte counts were not

Table 2. Changes in mean eosinophil counts and prevalence of eosinophilia in children: whole-school deworming programme (worm prevalence before and after deworming is summarized in the first footnote).

Occasion	Means (s.e.m.)[a]	P-values of changes in mean counts	Prevalence of eosinophilia[b]	Number of children
Before ℞[c]	0.612 (0.032)	–	47	200
After 2 ℞[d]	0.437 (0.032)	<0.01[d]	35	174
After 4 ℞[d]	0.377 (0.031)	<0.05[d]	23	197
After 6 ℞[d]	0.346 (0.029)	0.08[d]	24	256

Worm prevalence before and after deworming with mebendazole: *Trichuris* 72% and 14%; *Ascaris* 19% and 0%.

[a]S.e.m., standard error of the mean.

[b]Percentage of children with counts exceeding the normal paediatric range for eosinophils in venous blood: $0.05–0.45 \times 10^9$/l.[28,29]

[c]℞ = mebendazole 500 mg tablets (Vermox®, Janssen-Cilag): treatment intervals approximately four months. One tablet *per os*, compliance observed.

[d]Reduction in eosinophil counts was significant (P < 0.05) after both two and four dewormings. Further reduction after six dewormings approached significance.

influenced by the intervention. According to international criteria,[30] mebendazole at the 500 mg stat dose was highly effective against *Ascaris* in terms of cure rates and egg reduction rates and was moderately effective against *Trichuris* (data not shown).

Discussion

The children in both studies were infected by *Trichuris* and/or *Ascaris*, and no eggs of other helminths were detected in faecal samples that were obtained between four and six times from each child. *Trichuris* larvae and a permanently embedded portion of the adult worm are in prolonged contact with lymphoid tissue, mast cells and eosinophils in the intestinal mucosa.[13] *Ascaris* larvae undergo extensive tissue migration but adult worms probably have less direct contact with eosinophils.

Investigations concerning immune responses in endemic trichuriasis and ascariasis have reported on various immunological variables[7–12,24] but not on eosinophilia *per se*. Human trichuriasis seems to be associated with a mixed T-helper cell type 1 (Th1) and Th2 immune response,[12,13,24] while ascariasis is associated with Th2 polarization of the cytokine profile, which may be re-balanced by deworming.[7–9,11] Inappropriate Th2 bias during ascariasis, and infestation by some other worms, appears to be part of the reason for reduced efficacy of vaccines against various diseases, including cholera and tuberculosis.[5–26] The effectiveness of cholera and bacille Calmette-Guérin (BCG) vaccines can be restored to some extent by anthelminthic treatment.[7,11,17,18,20,21]

Activated eosinophils can attack stages of some helminths within tissues by releasing molecules that are cytotoxic to larvae.[14] Research using mice suggests that this kind of damage to larvae of *Strongyloides stercoralis* exposes antigens to which there may be an additional T-cell-dependent immune response.[31] This might apply in other helminthic infections as well. Immunoregulation by eosinophils can be via production of functional interleukin-13, which is a Th2 cytokine.[32]

Worm infestation and atopy can occur together[10,19,22,25] and eosinophilia is characteristic of both conditions.[10,15] The question of whether atopic disorders might be suppressed or exacerbated by anthelminthic treatment, in the presence of aeroallergens and sometimes genetic predisposition as well, has not yet been resolved. The relationship might vary with high and low endemicity of worm infestation, resulting in continuous or intermittent contact with helminthic antigens.[10,19,22,25]

Reversal of eosinophilia, which implies a degree of suppression of humoral immunity, was sustained in both studies at the whole-school level by means of regular treatment with benzimidazole anthelminthics. This result indicates that possible changes in other immune response markers should be researched, as well as the consequences of deworming in relation to co-endemic diseases. For immunological reasons in particular, deworming programmes could be relevant to the success of school and pre-school vaccination campaigns.[5–26,31,32]

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