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Helminth infection is associated with decreased basophil responsiveness in humans

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To the Editor

Helminth infections and allergic diseases are characterized by increases in IgE and type 2 cytokines such as IL-4. Despite their common immunopathogenesis, epidemiological studies reveal an inverse relationship between the prevalences of these diseases ^{1, 2} and a number of animal experiments have demonstrated that helminth infections can actively protect against the development of allergy ². While several mechanisms have been proposed, the pathophysiology underlying this phenomenon remains unclear ².

Recently, we demonstrated that chronic helminth infections suppress basophil responsiveness to IgE-mediated activation in mice ³. This phenomenon may be a principal mechanism underlying helminth-mediated protection against allergy as basophils are increasingly recognized as being functionally important in allergic diseases. Basophils participate in the effector phase of allergic responses by releasing acute inflammatory mediators such as histamine after IgE-mediated activation. Through the release of IL-4, they also play a prominent role amplifying the type 2 responses that drive allergic diseases ^{4, 5}. Given the many differences between murine and human basophils ^{5, 6}, in this study we

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sought to determine whether basophil suppression occurs in humans infected with helminths. To evaluate this, we compared basophil histamine release in helminth-infected children before and after anthelmintic treatment. Studies were approved by the Institutional Review Boards (IRBs) at the Universidad San Francisco de Quito, Ecuador, and at the Uniformed Services University (USU).

Parasitologic examinations were conducted on stool samples from twenty-eight children aged 8 – 14 years in a rural community in Esmeraldas Province, Ecuador. *Ascaris lumbricoides* and *Trichuris trichiura* eggs were found in the stool of all children and *Hymenolepis nana* eggs were found in the stool of 2 children. Blood from children was collected in heparinized tubes and centrifuged. After centrifugation, plasma was carefully removed and blood cells were washed twice with PBS. Blood cells were resuspended to the original volume using PBS, diluted with Histamine Release Buffer (Beckman Coulter, Inc.) and stimulated for 30 minutes with seven four-fold concentrations of anti-IgE (0.0005 µg/ml to 2 µg/ml, Sigma-Aldrich) and ionomycin (5 µg/ml, Calbiochem). Stimulated blood was centrifuged for 10 minutes at 400 × g, supernatant was acylated, and histamine levels determined using a competitive histamine ELISA (Beckman Coulter, Inc.). Percentage of total histamine released was calculated by dividing the amount of histamine released into supernatant by the amount of histamine in a lysed aliquot of blood. Infected children were then treated orally with three daily 800 mg doses of albendazole and a single dose of ivermectin at 0.2 mg/kg. Two weeks after therapy, histamine release from blood basophils was measured again from 22 of the treated children using identical stimulation conditions. To enable paired comparisons, only samples from the 22 children who provided blood before and after treatment were utilized in analyses. The two week timepoint was chosen as basophil lifespan is estimated to be 2 days⁷. Efforts were made to standardize blood processing, and basophil activation studies were conducted on average 4.5 hours after blood draw.

When sufficient plasma was available, samples were analyzed for circulating total IgE levels using a human IgE ELISA kit (Immunology Consultants Laboratory). Two-tailed Wilcoxon signed ranked test was used to determine statistical significance between paired samples. GraphPad Prism version 4.03 was used for all statistical analyses.

To evaluate whether helminth infections play a role in suppressing basophil function, histamine release from basophils of infected children was measured 2 weeks after anthelmintic therapy. As seen in figure 1, substantial increases in basophil activation in response to IgE-mediated activation developed in endemic children after their infections were treated. Two weeks after treatment basophils of endemic children released more of their total histamine in response to 0.031, 0.125, and 0.5 µg/ml anti-IgE (mean percentage of total histamine release, pre-treatment 32.8, 45.0, 42.1 % vs. post-treatment 46.9, 63.3, 57.7 % respectively $P=0.014$, $P=0.002$, $P=0.0007$, Fig. 1, A). These differences did not appear to be due to treatment-induced alterations in basophil IgE levels as no changes in circulating IgE were observed before and after treatment (Fig. 1, B).

To assess basophil responsiveness to non-IgE-mediated activation, histamine release was measured in response to ionomycin. As seen in figure 2A, anthelmintic treatment resulted in significantly greater basophil histamine release after ionomycin stimulation (mean percentage of total histamine release, pre-treatment 53.2 % vs. post-treatment 76.2 % $P=0.0005$). Of note, basal basophil histamine release was observed to be significantly lower post-treatment (mean concentration of spontaneous histamine release, pre-treatment 8.4 nM vs. post-treatment 2.1 nM $P=0.002$, Fig 2B), perhaps due to less ongoing activation of these cells in the absence of helminth infection. This phenomenon likely did not account for the observed increases in anti-IgE and ionomycin-mediated basophil activation post-

treatment, as we did not subtract out basal histamine release when calculating percentages of total histamine released. Additionally, analysis of individuals with equivalent baseline histamine release pre- and post-treatment exhibited the same changes as analysis of all individuals (data not shown).

These findings suggest that the ability of basophils to respond to both IgE-dependent and IgE-independent activation is suppressed during human intestinal helminth infection. This suppression appears to require the continuous presence of helminths as basophil histamine release increases within two weeks of the elimination of helminths. While the mechanism of basophil suppression requires further study, it could have important clinical consequences. First, as basophils have been shown to be important for protection against intestinal helminth infections in some murine models ⁸, basophil suppression may help worms to survive in hosts. Second, since basophils function as effector cells of allergy and are involved in the development of type 2 immune responses, reduced basophil functionality may be a mechanism by which helminths protect against allergic diseases.

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Abbreviations

IL-4	interleukin 4
PBS	Phosphate Buffered Saline
IgE	Immunoglobulin E
ELISA	Enzyme-Linked Immunosorbent Assay
mg	milligram
mg/kg	milligram per kilogram
wks (in figure legend)	weeks

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FIG 1.

Blood basophil histamine release in response to anti-IgE and IgE levels from infected children before and 2 weeks after anthelmintic treatment. **(A)**. Histamine released after stimulation with increasing concentrations of anti-IgE. **(B)**. Total IgE levels before and after treatment. Panels A, n=22 for pre and post-treatment groups. Panel B, n=14 for pre and post-treatment groups (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

FIG 2.

Blood basophil histamine release in response to ionomycin and spontaneous histamine release. **(A)**. Histamine released from basophils of infected children before and 2 wks after anthelmintic treatment after stimulation with 5 $\mu\text{g/ml}$ ionomycin. **(B)**. Spontaneous histamine released from basophils of helminth-infected children before and 2 wks after anthelmintic treatment. Panels A and B $n=22$ for pre and post-treatment groups (***, $P < 0.001$).