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# Do intestinal parasites enhance food allergy and atopic dermatitis?: A study in Warao Amerindian children

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**Keywords**
atopic dermatitis; food allergy; *Giardia duodenalis*; IgE; IL13; IL-6; TNF-α

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## Abstract

**Background:** We evaluated the influence of intestinal parasitic infection on food sensitization associated to the severity of Atopic Dermatitis (AD) in a group of Warao Amerindian pre- school children.
**Methods:** Feces examinations were performed in fresh stool specimens. Diagnosis of AD was done according to Hannifin and Rajka criteria and SCORAD index. Skin prick tests (SPT) were performed using extracts of cow's milk (CM), hen's egg (HE), *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* . Serum CM and HE-IgE levels (ELISA) were measured. Quantikine (R&D systems) assays were used for the determination of IL-13, TNF-α IL-6, and sCD23 in supernatants of CM- and HE- whole blood stimulated samples.
**Results:** Atopic Dermatitis was reported in 23% of the children. It was significantly ($p < 0.0001$) associated toward both CM and HE- SPT positivity ($p < 0.001$). *Giardia duodenalis* infection (37%) was associated to the presence of AD ($p = 0.005$) and to a significant increase in the levels of CM stimulated TNF-α ($p = 0.006$), IL-13 ($p = 0.01$), sCD23 ($p = 0.001$), CM-IgE ($p = 0.012$) and CM-SPT ($p < 0.0001$). Similarly, *G. duodenalis* infection was particularly associated with the increase on the levels of HE-stimulated TNF-α ($p = 0.001$), sCD23 ($p = 0.001$), HE-IgE levels ($p = 0.002$) and HE-SPT ($p = 0.001$).
**Conclusion:** Gut inflammation caused by *G. duodenalis* may enhance food allergic reactivity contributing to the manifestation of AD in these children. However, other environmental factors (not considered in this work) as well as an atopic background among the Warao population would also contribute to the presence of AD.

Atopic dermatitis (AD) is a common skin disease with worldwide prevalence rates of 1–20%. About 70% of the cases occur in children under fiveyears of age, with periods of remission and exacerbation of the disease affecting the quality of life of these patients (1). The immune mechanisms involved in the disease include activation and skin-selective homing of peripheral-blood T cells, mostly specific to food allergens, leading to the triggering of pro-inflammatory immune functions in the skin (2). CD4+ bearing the cutaneous-lymphocyte-associated antigen can induce the production of IgE, mainly via IL-13 (3) and prolong eosinophil lifespan through IL-5 activation (4). Also, several inflammatory cytokines like IL-6 (5) and TNF-α (6) have been associated to the severity of AD in human populations. In addition, dysregulated apoptosis in skin-homing T cells and keratinocytes contributes to the elicitation and progress of atopic dermatitis (7).

In Venezuela AD has been reported in around 9–12% of the pre -school children population, mostly urban (8, 9) being rare among rural populations (10, 11). However, recently, an increase on the prevalence of allergic diseases has been observed in rural children (12) including the Warao population (13).

The Amerindian Warao constitutes a population of around 21,000 people dispersed into isolated fishing and hunting communities along the delta of the Orinoco River in eastern Venezuela. They live in extreme poverty with very poor sanitary conditions and limited access to health assistance, which place the children at high biomedical risk (14). According to WHO resolutions (15), mass anthelmintic treatment chemotherapy has been implemented in all rural areas of Venezuela (16). This approach may reduce the prevalence and the intensity of these infections, improving life quality of the children (15, 16). However, routine programs for the control of protozoa infections have not yet been implemented. Thus, a high prevalence of giardiasis (around 35%) has been reported among distinct rural populations (17–19). Previous studies

have indicated that gut inflammation caused by *Giardia duodenalis* infection promotes the enhancement of sensitization towards food antigens favoring the development of allergic reactivity (20, 21). Taking these observations into account, we carried out a preliminary study to evaluate the prevalence and severity of AD in a group of Warao Amerindian pre- school children as well as the possible influence of *G. duodenalis* infection on the presence of food allergy associated to the manifestation of AD.

## Materials and methods

### Study population

We performed a cross-sectional study in an unselected group of 168 Warao Amerindian pre- school children (3–6 yr) belonging to the community of El Caigual located at the Delta Amacuro State, Venezuela. The community was selected according to the presence of a small health care center with facilities for clinical evaluation of the children, blood sampling and stool examination, and of a primary school that is attended by the majority of the children living in the community.

All pre-school children attending the primary school of the community, whose parents had signed the informed consent, were included in this study. The study was also approved by the Ethical Committee of the Institute of Biomedicine, Faculty of Medicine of the Central University of Venezuela. The population was sex balanced (88 girls and 80 boys) and with a mean age of 4.6 ± 1.2 yr.

### Stool examination

Three consecutive fresh stool specimens from each child were collected and examined microscopically for the presence of eggs, cysts, or larvae of intestinal parasites. The Kato–Katz (22) method was used for worm burden determinations.

### Clinical evaluation

The children were evaluated by a group of dermatologists – pediatricians from the Institute of Biomedicine, with the support of local physicians. Physical examination of the skin was performed using the criteria for diagnosis of atopic dermatitis established by Hannifin and Rajka (23) and assessment of severity depending on the SCORAD index (24). Skin prick tests (SPT) were carried using allergenic extracts of cow's milk, hen's egg, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* prepared as described previously (25) and at a concentration of 0.3 mg/ml in a saline solution with 50% glycerol and 0.4% phenol. Control tests were performed with the diluent alone and with 1% w/v histamine dihydrochloride. Wheal diameters were measured after 20 min and diameters ≥3 mm were considered as positive. Skin prick testing was carried out with appropriate facilities to treat systemic allergic reactions (anaphylaxis). Children under anti-allergic treatment were prevented to take oral antihistamine medication 10 days before application of the test.

### Food serum specific IgE levels

Specific anti-IgE levels toward extracts of commercial cow's milk proteins: casein, α-lactalbumin and β-lactoglobulin (Sigma–Aldrich) and commercial hen egg proteins: albumin and lysozyme from chicken egg white (Sigma–Aldrich, St Louis, MO, USA), were measured by an ELISA developed in our Laboratory and standardized against the commercial PHADIA -RAST technique. Briefly, 3 μg / well of the milk mixed-protein extract or 1.5 μg / well of the hen egg mixed-protein extract was coated onto 96-well microplates (Immunolon IV, Dynatech Laboratories Inc., El Paso, TX, USA) and incubated overnight at 4°C. Excess antigen was washed off with PBS-T and plates were blocked for two hours at 37°C with 1% BSA. Undiluted test sera were plated and incubated for 1 h at 37°C. After further washes with PBS-T, the plates were incubated with peroxidase-conjugated anti-human IgE diluted 1:1500 (Sigma-Aldrich). The washing process was repeated and o-phenylenediamine (OPD) plus $H_2O_2$ was added. The O.D. was read at 490 nm. A standard titration curve using a pool of sera with high specific anti cow' s milk – IgE or anti hen egg - IgE levels in the commercial PHADIA test was run with each assay, and the results were expressed as PHADIA RAST units (PRU/ml).

### Whole blood cultures and cytokine determinations

We carried out whole blood cultures following a protocol described previously (19). Briefly, whole blood samples of 5 ml of venous blood were collected from each child into lithium-heparin vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA). Four ml of whole blood was diluted 1:4 with RPMI 1640 medium (SIGMA) supplemented with 2 mM l-glutamine (GIBCO, Life Technologies, Grand Island, NY, USA) and gentamicin (80 μg/ml; GIBCO). 1 ml of the blood suspension was plated in 48 –well plates and stimulated with antigen extract at a previously determined optimal concentration: 12.5 μg/ml for the extract of a mixture of milk proteins: α-casein, β-casein, α-lactalbumin, β-lactoglobulin (Sigma–Aldrich) or 8.5 μg/ml of an extract of a mixture of hen egg proteins: albumin and lysosyme from chicken egg white (Sigma–Aldrich) and 5 μg/ml of phytohemagglutinin (PHA) (SIGMA). A third well was un-stimulated. All plates were incubated for 48 h at 37°C with 5% $CO_2$. Supernatants were removed, centrifuged at 1500 ***g*** for 15 min and were stored at −70°C for future assays.

### Cytokine determinations

The Quantikine cytokine assay (R&D Systems, Minneapolis, MN, USA) was used for the determination of IL-13, TNF-α IL-6, and sCD23 in samples of cow's milk and hen egg -stimulated whole blood-supernatants. The sensitivity of the assay was of 32 pg/ml for IL-13; 1.6 pg/ml for TNF-α, 0, 7 pg/ml for IL-6 and 3, 18 pg/ml for sCD23 determinations. Responses to PHA and un-stimulated supernatants were recorded (data not shown) but statistical analysis was performed only in antigen- specific cytokine levels. The value corresponding to each un-stimulated well was subtracted from each respective PHA and specific cytokine measurement.

### Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 5.00 for Windows, GraphPad Software (San Diego, CA, USA). Fisher's exact Test was used to evaluate the associations between sex, parasitic infection, and skin allergic reactivity with the presence of atopic dermatitis as well as the association between skin allergic reactivity with *G. duodenalis* infection. Non-adjusted (crude) odds ratio values as well as 95% confidence interval are shown for each comparison in the results section and in the respective tables.

The Mann–Whitney Test was used to compare the medians of age and of the levels of IgE antibodies and cytokines according to the presence of atopic dermatitis. Spearman rank correlations were used to study the associations between the SCORAD index as well as of the presence of *G. duodenalis* infection with the levels of IgE and cytokines.

## Results

### Prevalence of intestinal parasitic infections

A moderate prevalence of intestinal helminths (23.5%) was found, being *Ascaris lumbricoides* (21.3%) and *Trichuris trichiura* (19.2%) the most frequent parasites. According to the WHO classification (22), low intensities of these infections (median of *A. lumbricoides* worm burden: 500 eggs/g. feces; median of *T. trichiuris* worm burden: 370 egg /g. feces) were observed. The prevalence of protozoa intestinal parasites was of 35.7% for *G. duodenalis*, 39.8% for *Blastocystis hominis* and 6% of *Entamoeba histolitica/dyspar*.

### Clinical evaluation

It was found that 23% of the children exhibited at least one symptom associated to the presence of atopic dermatitis (Table 1). Thus, 21.5% of the children manifested skin xerosis accompanied by pruritus. In addition, face and/or neck erythema was observed in 16.5% of the children. Erythema with excoriations comprising extensor surfaces of extremities was found in 12% of the children and 8.5% exhibited flexural lichenified dermatitis. The median of the SCORAD among these children was of 13 points; the 75th percentile of the SCORAD was of 25.5 points and the 25th percentile of 6.25 points. A significant prevalence of SPT positivity (29.1%) was found among these children (Table 1) being SPT positivity toward cow milk extract of 28% and that of hen egg extract of 17%. SPT positivity toward aero-allergens such as *D. pteronyssinus* and *D. farinae* were under 12%.

### Associations between immune parameters and atopic dermatitis

We did not found any association of sex ($p = 0.588$; odds ratio: 0.79; 95% CI: 0.39–1.62) and age (Mann–Whitney U = 2106; $p = 0.272$) with the presence of AD among this group of children.

Skin Prick test (SPT) toward cow milk and hen egg extracts were strongly associated ($p < 0.0001$) with the presence of AD whereas SPT toward aero allergens such as *D. pteronyssinus* and *D. farinae* were not significantly associated (Table 2).

**Table 1** Allergic reactivity and atopic dermatitis in a group of Warao Amerindian pre-school children (n = 168)

| | Prevalence (%) |
|---|---|
| Atopic dermatitis* | 23.2 |
| Skin xerosis + Pruritus | 21.5 |
| Erythema (face and neck) | 16.5 |
| Erythema with excorations(extensor surfaces of extremities) | 12 |
| Flexural lichenified dermatitis | 8.0 |
| Skin Allergic reactivity† | 29.1 |
| Cow milk SPT | 28.0 |
| Hen egg SPT positivity | 17.0 |
| *Dermatophagoides pteronyssinus* SPT positivity | 10.5 |
| *Dermatophagoides farinae* SPT positivity | 9.6 |

*% of children with at least one symptom of atopic dermatitis according to the criteria of Haniffin & Rajka.
†% of children with at least two positive skin prick test (wheal ≥3 mm).

The median of the levels of serum IgE against cow milk and hen egg extracts were significantly elevated ($p < 0.001$) in those children with AD compared with those without symptoms of AD. Similarly, the median of the levels of cow milk and hen egg-stimulated cytokines (sCD23, IL-6, IL-13 and TNF-α) were significantly higher ($p > 0.0001$) in the group of children with AD compared with those without AD (Table 3).

Spearman rank correlations between the SCORAD index and cytokines showed that cow milk-stimulated TNF-α cytokine levels were strongly associated with the severity of atopic dermatitis ($p = 0.009$) while it was only slightly associated with cow milk-stimulated IL-6 ($p = 0.02$), IL-13 ($p = 0.03$) and sCD23 ($p = 0.026$) cytokines levels (Table 4). Also, significant associations were observed between the SCORAD index and hen egg-stimulated IL6 ($p = 0.0005$), IL-13 ($p = 0.0015$), TNF-α ($p = 0.0004$), and sCD23 ($p = 0.0054$) cytokines. The levels of serum IgE against cow milk and hen egg allergen extracts were found to be significantly associated ($p = 0.002$ and $p = 0.0001$ respectively) to the severity of AD (Table 4).

### Influence of intestinal parasitic infection on the prevalence and severity of AD

Infection by *G. duodenalis* was positively associated ($p = 0.005$; odds ratio: 4.79; 95% CI: 2.24–10.26) to the presence of AD while the presence of other intestinal parasites such as *B. hominis* ($p = 0.496$; odds ratio: 0.487; 95% CI: 0.05–4.83) or *A. lumbricoides* ($p = 0.54$; odds ratio: 1.26; CI: 0.46–4.52) did not affect the prevalence of this disease. In addition, significant associations were found between infection by *G. duodenalis* with cow milk-SPT ($p = 0.0006$) and hen egg-SPT ($p = 0.019$). There were no associations between *G. duodenalis* infection and SPT positivity toward aeroallergens (Table 5).

 

**Table 2** Association between skin allergic reactivity and atopic dermatitis in a group of Warao Amerindian pre-school children

| | With atopic dermatitis (39) | Without atopic dermatitis (129) | Statistical significance (p) | Odds ratio | 95% CI |
|---|---|---|---|---|---|
| % SPT Cow milk* | 76.9 | 7.5 | $p < 0.0001$ | 44.40 | 16.23–121.7 |
| % SPT Hen egg* | 48.71 | 7.75 | $p < 0.0001$ | 11.31 | 4.59–27.82 |
| % SP*T Dermatophagoides pteronyssinus** | 10.25 | 10.82 | $p = 1.00$ | 0.938 | 0.29–3.07 |
| % SPT *Dermatophagoides farina** | 7.69 | 10.07 | $p = 1.05$ | 0.743 | 0.20–2.75 |

*Wheal ≥3 mm.

The presence of *G. duodenalis* was found to be associated to a significant increase on the levels of cow milk-stimulated TNF- α ($p = 0.0061$), IL-13 ($p = 0.01$) and sCD23 ($p = 0.0014$) cytokines as well as to an increase on the levels of serum cow milk-IgE ($p = 0.01$). Similarly there was a significant association between *G. duodenalis* infection and the increase of the levels of hen egg-stimulated TNF-α ($p = 0.001$), IL-13 ($p = 0.015$), and sCD23 ($p = 0.0017$) cytokines and with the increase of the levels of serum hen egg- IgE ($p = 0.023$) (Table 6).

## Discussion

In this study, we found a high prevalence of atopic dermatitis in a group of rural pre-school children belonging to the Warao ethnic group; this prevalence being noticeable higher than that previously reported in other groups of Venezuelan children (8, 9).

The presence of AD was associated with a high SPT positivity as well as elevated levels of IgE toward cow milk and hen egg proteins extracts. Even though cow milk and hen eggs are not frequent as part of the traditional food of this ethnic group (14), it is possible that westernization has been gradually changing alimentary practices, introducing new sources of proteins to the traditional Warao diet. In addition, the presence of a school dining in the community offering three meals a day, according to the Venezuelan national school alimentary program (26), would contribute to the exposure of Warao atopic children to potential food allergens. However, further studies are needed to elucidate the associations between alimentary practices, diet components and allergic reactivity among these children. In contrast, probably due to their particular life style (14) exposure to house dust among these children would be rare (11), thereby exhibiting low SPT positivity against *Dermatophagoydes* extracts.

**Table 3** Immunological parameters according to the presence of atopic dermatitis in a group of Warao Amerindian pre-school children

| | Cow milk | | | Hen egg | | |
|---|---|---|---|---|---|---|
| | With atopic dermatitis (39) | Without atopic dermatitis (129) | Mann–Whitney U (statistical significance) | With atopic dermatitis (39) | Without atopic dermatitis (129) | Mann–Whitney U (statistical significance) |
| IgE UI/ml | | | | | | |
| 25% percentile | 0.75 | 0.15 | 149.0 ($p < 0.0001$) | 0.3 | 0.12 | 298.5 ($p < 0.0001$) |
| Median | 0.85 | 0.25 | | 0.5 | 0.2 | |
| 75% percentile | 0.9 | 0.3 | | 0.65 | 0.25 | |
| sCD23 pg/ml* | | | | | | |
| 25% percentile | 175 | 85 | 480.5 ($p < 0.0001$) | 155 | 55 | 193.1 ($p < 0.0001$) |
| Median | 250 | 125 | | 180 | 115 | |
| 75% percentile | 355 | 150 | | 230 | 125 | |
| IL6 pg/ml* | | | | | | |
| 25% percentile | 5.5 | 0.18 | 140 ($p < 0.0001$) | 4.5 | 0.19 | 175 ($p < 0.0001$) |
| Median | 7.6 | 0.35 | | 5.7 | 0.33 | |
| 75% percentile | 8.3 | 0.74 | | 6.7 | 0.55 | |
| IL-13 pg/ml* | | | | | | |
| 25% percentile | 240 | 35.4 | 157 ($p < 0.0001$) | 240 | 25.7 | 48 ($p < 0.001$) |
| Median | 400 | 80 | | 340 | 56 | |
| 75% percentile | 480 | 125 | | 430 | 81.5 | |
| TNF-α pg/ml* | | | | | | |
| 25% percentile | 142 | 25.7 | 214 ($p < 0.0001$) | 120 | 25.2 | 263.5 ($p < 0.0001$) |
| Median | 165 | 65 | | 145 | 45.5 | |
| 75% percentile | 180 | 80.2 | | 155 | 67.6 | |

*Cytokines from whole blood-supernatants stimulated with 12.5 μg/ml of an extract of milk proteins: α-casein, β-casein, $\tilde{\alpha}$ lactoalbumin, $\tilde{\beta}$ lactoalbumin or with 8.5 μg/ml of an extract of hen egg proteins: albumin from chicken egg white and lysozyme from chicken egg white. N = 168.

The association of food allergic reactivity with the presence of AD was accompanied by a strong elevation of cow milk- and hen egg-stimulated IL-13 plasma levels. Previous studies have indicated that IL-13 is involved in the stimulation of IgE in patients with AD (3) and high levels of peripheral CD4+ IL-4+ and CD4+ IL-13+ T cells have shown to be associated with

**Table 4** Associations of the severity of atopic dermatitis (scorad) with different immunological parameters in a group of Warao Amerindian pre-school children with scorad ≥3.5

| | IL-6 pg/ml* | IL-13 pg/ml* | TNF-α pg/ml* | IgE PRU/ml | sCD23 pg/ml |
|---|---|---|---|---|---|
| Number of XY Pairs | 51 | 51 | 51 | 51 | 51 |
| Cow milk | | | | | |
| Spearman r | 0.306 | 0.302 | 0.361 | 0.430 | 0.287 |
| 95% confidence interval | 0.025–0.542 | 0.021–0.539 | 0.087–0.585 | 0.167–0.360 | 0.201–0.512 |
| p Value (two-tailed) | 0.028 | 0.030 | 0.009 | 0.002 | 0.026 |
| p Value summary | _* | _* | _** | _** | _* |
| Hen egg | | | | | |
| Spearman r | 0.469 | 0.434 | 0.477 | 0.652 | 0.384 |
| 95% confidence interval | 0.214–0.664 | 0.171–0.638 | 0.224–0.670 | 0.452–0.789 | 0.113–0.601 |
| p Value (two-tailed) | 0.0005 | 0.0015 | 0.0004 | 0.0001 | 0.0054 |
| p Value summary | _*** | _** | _*** | _*** | _** |

*Cytokines from whole blood-supernatants stimulated with 12.5 μg/ml of an extract of milk proteins: α-casein, β-casein, $\tilde{\alpha}$ lactoalbumin, $\tilde{\beta}$ lactoalbumin or with 8.5 μg/ml of an extract of hen egg proteins: albumin from chicken egg white and lysozyme from chicken egg white.
**Significant.
***Extremely significant.

**Table 5** Association between skin allergic reactivity and at the presence of *Giardia Duodenalis* in a group of Warao Amerindian pre-school children

| | Parasitized with *G. duodenalis* (60) | Non-parasitized (108) | Statistical significance (p) | Odds Ratio | 95% CI |
|---|---|---|---|---|---|
| % SPT Cow milk* | 45.1 | 18.5 | p = 0.0006 | 3.6 | 1.78–7.27 |
| % SPT Hen egg* | 26.6 | 12.0 | p = 0.019 | 2.65 | 1.17–6.0 |
| % SP*T Dermatophagoides pteronyssinus** | 16.6 | 7.4 | p = 0.07 | 0.93 | 0.92–6.72 |
| % SPT *Dermatophagoides farinae** | 15.0 | 6.4 | p = 0.098 | 2.54 | 0.89–7.23 |

*Wheal ≥3 mm.

**Table 6** Associations of the presence of *Giardia duodenalis* with different immunological parameters in a group of Warao Amerindian pre-school children

| | IL-6 pg/ml[1] | IL-13 pg/ml* | TNF-α pg/ml* | IgE PRU/ml | sCD23 pg/ml |
|---|---|---|---|---|---|
| Number of XY Pairs | 168 | 168 | 168 | 168 | 168 |
| Cow milk | | | | | |
| Spearman r | 0.1482 | 0.1982 | 0.2108 | 0.1922 | 0.235 |
| 95% confidence interval | −0.0078–0.297 | 0.0437–0.343 | 0.0568–0.355 | 0.037–0.338 | 0.075–0.292 |
| p Value (two-tailed) | 0.0552 | 0.01 | 0.0061 | 0.0126 | 0.0014 |
| p Value summary | ns | _* | _** | _* | _** |
| Hen egg | | | | | |
| Spearman r | 0.129 | 0.187 | 0.240 | 0.174 | 0.240 |
| 95% confidence interval | −0.027–0.28 | 0.031–0.33 | 0.08–0.38 | 0.019–0.03 | 0.087–0.321 |
| p Value (two-tailed) | 0.094 | 0.015 | 0.001 | 0.023 | 0.0017 |
| p Value summary | ns | _* | _** | _* | _** |

[1]Cytokines from whole blood-supernatants stimulated with 12.5 μg/ml of an extract of milk proteins: α-casein, β-casein, $\tilde{\alpha}$ lactoalbumin, $\tilde{\beta}$ lactoalbumin or with 8.5 μg/ml of an extract of hen egg proteins: albumin from chicken egg white and lysozyme from chicken egg white.
*Slightly significant.
**Significant.

 

the severity of AD in children (27). Besides, an association of cow milk and hen egg-stimulated TNF-levels with the severity of AD was also observed confirming previous findings from other studies in human populations (6). It has been reported that this cytokine is able to induce the expression of several adhesion molecules including intercellular adhesion molecule-1 (ICAM-1), which has been considered to be an important initiator of leucocyte/keratinocyte interactions in skin inflammation (28). However, other factors leading to the disruption of epidermal skin barrier (5, 7) which would also contribute to the development of AD in these children must be investigated.

On the other hand, gut mucosa homeostasis has been considered as a key factor in preventing the development of food allergy (29). In this work, the presence of *G. duodenalis* was associated to an increase of the levels of IgE and pro-inflammatory cytokines toward food allergens. There is evidence that *Giardia* disrupts enterocyte α-actinin, a component of the actomyosin ring that regulates paracellular flow across intestinal epithelia (30). The parasite may also alter members of the claudin protein family, a critical component of the sealing properties of tight junctions (31) indicating that in giardiasis, changes in the structure and function of the enterocytes are associated with a loss of intestinal epithelial barrier function which may in turn favor the traffic of allergenic macromolecules through damaged epithelia that would favor the development of AD. However, it has been reported that other mechanisms such as the reduction of the composition of the gut microflora, may also increase the risk of AD (32). In addition, along with an atopic background among these children (33), exposure to particular components of the early life environment, may affect both local development of regulatory components of the mucosal immune system and immune responses to food proteins (34), thus influencing the outcome of this disease. These important aspects would be the subject of our future studies.

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