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Short Communication

Severity of atopic dermatitis and *Ascaris lumbricoides* infection: an evaluation of CCR4+ and CXCR3+ helper T cell frequency

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ABSTRACT

Introduction: *Ascaris lumbricoides*-infected patients present lower prevalence of severe atopic dermatitis. **Methods:** Peripheral blood of infected children with atopic dermatitis was assessed by flow cytometry of the frequency of Th1 and Th2 cells through the expression of CXCR3 and CCR4 chemokine receptors, respectively. **Results:** Helminth-free patients with atopic dermatitis presented a high frequency of CCR4+Th2 cells. Parasitized patients with atopic dermatitis showed a lower frequency of CXCR3+Th1 cells compared to infected individuals only. **Conclusions:** *Ascariasis* modifies the blood traffic of Th2 cells in atopic dermatitis patients, while the allergic disease down-regulates the traffic of Th1 cells in parasitized patients.

Keywords: Atopic dermatitis. *Ascaris lumbricoides*. CCR4 and CXCR3 receptors.

Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with genetic background and environmental factors[1]. The course of the disease is marked by biphasic clinical manifestations, which are closely related to the selective trafficking of both Th2 and Th1 cells from peripheral blood towards the skin by the expression of adhesion molecules and chemokine receptors[1]. Thus, in acute AD, patients present IL-4 production together with a high frequency of CCR4+Th2 cells, which is related to the severity of the disease[2]. Subsequently, IFN-γ was detected in the chronic phase, reflecting the predominance of CXCR3+Th1 cells[3].

A strong inverse association between atopy and active helminth infections has been demonstrated[4]. With regard to AD symptoms, Cooper et al.[5] have pointed out that the presence of geohelminth infections does not offer protection against atopic eczema. In contrast, it was demonstrated that previous or current *A. lumbricoides* infections can reduce the frequency of atopic eczema in schoolchildren[6,7]. Recently, we have observed that ascariasis decreased the frequency of severe dermatitis in children from an urban area endemic for this helminthiasis[8]. In this study, we investigated the frequency of CCR4+Th2 and CXCR3+Th1 cells in the peripheral blood of *A. lumbricoides*-infected AD children.

The study was performed in Pedregal, an area of low socioeconomic index in the City of Campina Grande, Brazil, where a high AD prevalence has been observed[8]. All parents or guardians of the children (aged 2 to 10 years), after providing written consent to take part in the study, were asked about AD symptoms through the questionnaire of the International Study of Asthma and Allergies in Childhood (ISAAC)[9]. Clinical data and feces samples (Ritchie; Kato-Katz methods) were collected over a period of 18 months. All the children with positive results for geohelminth infection were duly treated with mebendazole. The AD children were recalled for a second clinical and parasitological examination.

Fifty-four children were selected after they had fulfilled the clinical and parasitological conditions of the established groups: *A. lumbricoides*-infected children (ASC, n = 10); *A. lumbricoides*-infected and AD children (ASC+AD, n=12); AD children (AD, n=22); and the control group (noninfected and non-AD children (NI+NAD, n=9). Double labeling was performed in peripheral blood mononuclear cells (PBMC) ($1x10^6$) from each child. The PBMC were obtained by centrifugation in Ficoll-Hypaque reagent (Sigma-Aldrich) after incubation with fluorochrome-conjugated antibodies (e-Bioscience, CA, USA): PE-Cy5 anti-human CD4 plus PE anti-human CCR4 or PE anti-human CXCR3, for 30min at room temperature. After washing, the PBMC were analyzed in a Guava personal cell analyzer (PCA) flow cytometer (GE). Limits for the quadrant markers were set based on negative populations. All values were expressed as mean ± SEM. Statistical differences ($p < 0.05$) between groups were assessed using Kruskal-Wallis test and Dunn's post-test. The study was approved by the Ethics Committee on Human Research of the Hospital Universitário Alcides Carneiro, Universidade Federal de Campina Grande - PB.

There was no difference regarding age (7 ± 2.5 years) and gender among groups. A low parasite load was observed in all parasitized children. **Figure 1A** shows a higher frequency of CCR4+CD4+ cells in noninfected AD patients when compared to the control group (Non-infected + Non-AD). **Figure 1B** depicts the CXCR3+CD4+ cell frequency, which was significantly higher in infected children only.

Epidemiological studies on the association between worm infestation and eczema are scarce[5-8]. For the first time, our results showed that current *A. lumbricoides* infections in AD children from an urban area decreased the risk of severe AD symptoms[8]. We showed in this study that infected AD patients present a low frequency of CCR4+ lymphocytes, a membrane marker involved in Th2 cell chemotactic influx.

This study was performed in an endemic area for *A. lumbricoides*[8]. However, the mass treatment, as well as the total of dropouts during the course of the study, limited the number of reinfected AD patients in

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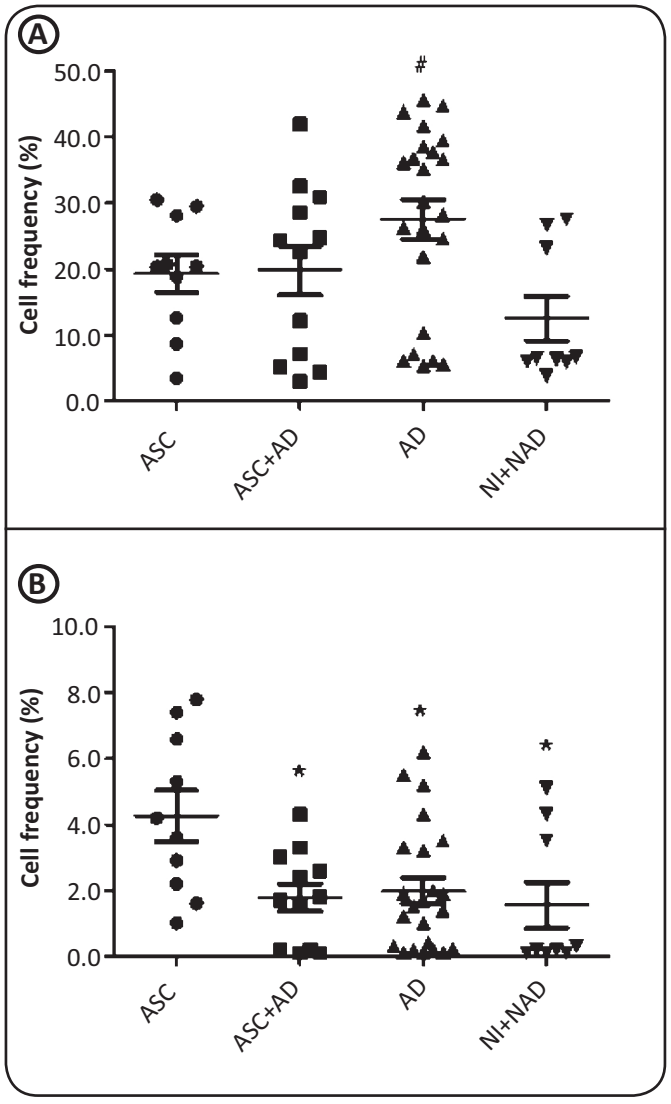

FIGURE 1 - CCR4+CD4+ (A) and CXCR3+CD4+ (B) cells in the peripheral blood of *A. lumbricoides*-infected and atopic dermatitis (ASC+AD), helminth-free atopic dermatitis (AD), *A. lumbricoides*-infected (ASC) only, and noninfected or non-atopic dermatitis (NI+NAD) patients. Cells (1 x 10^6) were stained with anti-CCR4-PE or anti-CXCR3-PE and anti-CD4-PE-Cy5 monoclonal antibodies and analyzed by flow cytometry. The results correspond to the percentage of double-positive cells. #p < 0.05 compared to the NI+NAD group. *p < 0.05 compared to the ASC group.

this group. Hence, two patients from the ASC group and three from the ASC+AD group harboring concurrent *Trichuris trichiura* infection were not excluded. We believe that the coinfection did not interfere with our results since intragroup analyses showed similar frequencies of cells in coinfected patients and in those infected with *A. lumbricoides* only.

Ascariasis and AD are diseases in which the effector mechanisms are elicited by the Th2-type immune response (IgE, IL-4, IL-5, and eosinophilia)[4]. Indeed, the children without AD or helminthes had a low frequency of CCR4+CD4+ cells in their blood. We are aware that patients submitted to long-term treatment with the imidazolidine compound may have an increased Th2 response to *A. lumbricoides*[10]. In our study, the short-term treatment would not affect the Th2 cytokine production in response to the parasite or allergens.

Moreover, in the helminth-free AD children, the CCR4+CD4+ cell profile was quite evident. This finding suggests that without concurrent infection, there is a higher Th2 clone expansion or an enhanced mechanism of activated Th2 cell attraction in the peripheral blood. Recent investigations have demonstrated the generation of regulatory T (Treg) cells during ascariasis. Treg cells were associated with down-regulation of the allergen-specific Th2 response in the host, regardless of the IL-10 cytokine[11,12]. Whether Treg cells and/or suppressive cytokines are involved in the lower frequency of Th2 cells in the peripheral blood of infected AD patients requires further experimental analysis.

A lower CXCR3+Th1 cell frequency was observed in ASC+AD patients compared to the ASC group. This result suggests a significant down-regulatory effect of atopic dermatitis status on Th1 response in the helminth infection. Furthermore, this finding strengthens the hypothesis that the atopic phenotype induces resistance to geohelminth infection[13]. Indeed, active ascariasis was associated with diminished Th2 responses to helminth antigens, whereas atopic children had greater frequencies of PBMCs expressing IL-4 and IL-5 compared to nonatopic children[13].

In conclusion, our findings showed a high frequency of CCR4+Th2 cells in the peripheral blood of *A. lumbricoides*-free AD patients, in agreement with the high propensity for severe skin symptoms[8]. Besides, the finding that atopic dermatitis lowered the blood traffic of Th1 cells in the concomitant infected patients suggested that ascariasis and atopic phenotype can influence each other, modifying the blood traffic of Th2 and Th1 in patients with both diseases.

ABSTRACT IN PORTUGUESE

Gravidade da dermatite atópica e infecção por *Ascaris lumbricoides:* uma avaliação da frequência de células T auxiliares CCR4+ e CXCR3+

Introdução: Pacientes infectados com *Ascaris lumbricoides* apresentam menor prevalência de dermatite atópica grave. **Métodos:** Sangue periférico de crianças infectadas com dermatite atópica foi analisado por citometria de fluxo quanto à frequência de células Th1 e Th2 pela expressão de receptores de quimiocina CXCR3 e CCR4, respectivamente. **Resultados:** Pacientes sem helmintos com dermatite atópica apresentaram alta frequência de células Th2CCR4+. Pacientes parasitados com dermatite atópica apresentaram menor frequência de células Th1CXCR3+ comparados aos indivíduos apenas infectados. **Conclusões:** Ascaridiases altera o tráfego sanguíneo de células Th2 em pacientes com dermatite atópica, enquanto a doença alérgica diminui o tráfego de células Th1 em pacientes parasitados.

Palavras-chaves: Dermatite atópica. *Ascaris lumbricoides.* Receptores CCR4 e CXCR3.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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