

Helminth extracts inhibit eosinophilic inflammation in a murine model of allergic rhinitis

To the Editor,

Allergic rhinitis (AR) is highly prevalent in many populations, resulting in considerable morbidity and socioeconomic costs.¹ Inflammation in AR is mainly characterised by inflammatory response with eosinophil recruitment and activation in nasal mucosa.² Although the pathophysiology of allergic diseases has been widely studied, effective primary prevention treatments are not available.

Previous studies have shown that in developing countries, where helminth infections have a high prevalence, atopy airway reactivity is negatively associated with parasite infections.^{3,4} In experimental models, mice exposed to different helminths have consistently shown that allergic pulmonary response to ovalbumin (OVA) was significantly inhibited.^{5,6} One important question raised is whether the upper airways may also be influenced by helminth exposure. This is the first study to assess the effect of helminths in a model of AR. The aim of this short report is to analyse the effect of the exposure to different parasite extracts on the influx of eosinophils in the nasal mucosa of mice with upper airway eosinophilic response to OVA. Female BALB/c mice (6–8) weeks old were used. Mice ($n=45$) were divided into four groups, according to the helminths they were exposed to: *Angiostrongylus costaricensis* ($n=12$); *Angiostrongylus cantonensis* ($n=13$); *Ascaris lumbricoides* ($n=14$); and one control group ($n=6$). Animals were housed in cages with filter-cap and in a temperature-controlled room at the animal house, with water and food *ad libitum* and kept on a 12-h light/dark cycle.

Adult worms were obtained from the Laboratory of Molecular Parasitology (Institute of Biomedical Research/PUCRS). The extract of worms was carefully soaked and washed separately and liquid nitrogen was then added to perform lyophilisation. Buffered solution of TRIS NaCl 20 mM with protease inhibitors (TLCK, EDTA, PMSF) was used as the extraction buffer. The resulting solution was sonicated three times for 2 min and then centrifuged twice (12,000 rpm/1000 \times g) for 20 min at 4 °C. Supernatant was extracted and the protein concentration was estimated using the method of Bradford (Bio-Rad protein assay, USA). The concentration for intraperitoneal (i.p.) injection of protein extract was 340 μ g/200 μ L for each mouse of the groups studied. Mice were exposed to parasite extract seven days prior to OVA sensitisation.

Mice were sensitised by two i.p injections of 100 μ g of OVA (grade V, Sigma, USA), with 100 μ g of alum (aluminium hydroxide hydrate, Sigma, USA), diluted in phosphate-buffered saline (PBS, total volume: 200 μ L) on days 0 and 14. Ovalbumin instillation (100 μ g of OVA, diluted in 50 μ L of PBS) was micropipetted into both nostrils of each mouse, from days 25 to 27, under light anaesthesia with halothane. For histological analysis, mice were euthanised 24 h after the last nasal challenge, with high ketamine/xylazine dosage injections.

In all groups, heads were separated from the body, and scalp and lower jaw were removed. Heads were fixed in 10% buffered formalin for at least 48 h. After fixation, heads were decalcified in 12% ethylenediaminetetraacetic acid solution (EDTA, Sigma, USA), for two weeks. After decalcification, heads were cut through coronal sections into four regions for paraffin embedding: (A) at the incisor teeth; (B) halfway between A and C; (C) at the anterior margin of orbit; and (D) halfway between the posterior margin of the orbit and C. Tissue sections were cut at 3–4 μ m thickness and stained with haematoxylin–eosin (H&E).

The number of eosinophils was quantified per area (mm^2) within the submucosa or mucosa of the septum or turbinates, encompassing an area of 0.0165 mm^2 , using the software Image Pro 4.5[®]. The average of cells counted in six high-power fields (400 \times) was used for data analysis. Slides were examined by two independent observers, who were blinded to the animal groups analysed.

Data were analysed with SPSS 11.5 for Windows (SPSS Inc., USA). Data were expressed as mean \pm SEM. The mean eosinophil counts analysed were compared among groups using one-way analysis of variance (ANOVA) test, with Bonferroni test. Differences were considered to be statistically significant when $p < 0.05$. This study was approved by the Animal Ethics Committee from our institution.

Mice from the positive control group, sensitised and challenged with OVA, developed significant upper airway eosinophil infiltration. The lamina propria was thickened, and inflammatory cells and oedema of the nasal mucosa were more intense in the positive control group. Eosinophil infiltration was almost exclusively confined to the underlying submucosa. Eosinophil infiltration was most intense in sections C and D within the lower one-half of the nasal septum and lateral nasal wall. All mice that were previously exposed to parasite extracts had very low degree of eosinophil infiltration in the submucosa (Fig. 1), and less mucosal oedema. The mean of eosinophil cell counts from the *A. costaricensis* group was 1.88 ± 0.60 cells/ mm^2 . *A. lumbricoides* and *A. cantonensis* eosinophil cell counts were respectively 2.37 ± 0.66 cells/ mm^2 and 2.31 ± 0.56 cells/ mm^2 . Eosinophil infiltration in parasite extract groups was significantly suppressed when compared to the control group ($p = 0.001$; Fig. 2). Eosinophil cell counts were not significantly different among the parasite extract groups.

We demonstrated that mice exposed systemically to distinct parasite extracts before sensitisation and challenge to OVA showed a significant suppression of eosinophilic inflammation in the upper airways, showing less non-specific inflammatory abnormalities (oedema of mucosa). These original findings offer a new approach to explain the relationship between helminths and allergic diseases.

Previous studies have focused on the interaction between the parasite infection process during the natural parasite cycle in the host and the development of atopic asthma in humans.^{3,4} Other reports have shown in animal models that helminths inhibit allergic pulmonary response.^{5–7} We have previously demonstrated that *A. costaricensis* infection and exposure to its extract were both associated with a decrease

striking localised upper airway infiltration with eosinophils, showing that this is an adequate model to study upper airway eosinophilic response to allergens and the effect of interventions.

Our main outcome is supported by eosinophil quantification per area of a sample of upper airway tissue. Eosinophils are one of the pivotal cells playing a role in the pathophysiology of allergic rhinitis. In the same line as the study by Okano et al., which used eosinophil counts as a primary outcome in a model of AR², the authors believe that the eosinophil count analysis can be used to support our preliminary results and conclusions. It seems clear from our study that the tested helminth extracts significantly suppress eosinophil response in this allergic rhinitis model. However, more precise mechanistic analysis between helminth exposure and the development of allergy is required in further studies in order to better understand this potent inhibitory effect, such as specific IgE and T cell regulatory responses.

In conclusion, this is the first study to show that exposure of parasite extracts of adult *A. costaricensis*, *A. cantonensis* and *A. lumbricoides* worms strongly inhibits eosinophilic inflammatory response in a murine model of allergic rhinitis. Helminths seem to strongly inhibit the upper airway eosinophilic allergic response, and hence, our data highlight the importance of studying in more detail helminth bioproducts in order to find effective alternatives to inhibit the immune response against allergens in predisposed individuals, regardless of the heterogeneous constituent of any potential new therapy, which is a central part of the translational component of our field of research.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

References

1. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63 Suppl. 86:8–160.
2. Okano M, Nishizaki K, Abe M, Wang MM, Yoshino T, Satoskar AR, et al. Strain-dependent induction of allergic rhinitis without adjuvant in mice. *Allergy*. 1999;54:593–601.
3. van den Biggelaar AH, van Ree R, Rodrigues LC, Lell B, Deelder AM, Kremsner PG, et al. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet*. 2000;356:1723–7.
4. Hagel I, Lynch NR, Perez M, Di Prisco MC, Lopez R, Rojas E. Modulation of the allergic reactivity of slum children by helminthic infection. *Parasite Immunol*. 1993;15:311–5.
5. Wang CC, Nolan TJ, Schad GA, Abraham D. Infection of mice with the helminth *Strongyloides stercoralis* suppresses pulmonary allergic responses to ovalbumin. *Clin Exp Allergy*. 2001;31:495–503.
6. Pinto LA, Dias AC, Rymer BL, Fernandes FF, Barbosa GL, Machado DC, et al. Effect of *Angiostrongylus costaricensis* extract on eosinophilic pulmonary response in BALB/c mice. *Parasitol Res*. 2006;98:295–8.
7. Trujillo-Vargas CM, Werner-Klein M, Wohlleben G, Polte T, Hansen G, Ehlers S, et al. Helminth-derived products inhibit the development of allergic responses in mice. *Am J Respir Crit Care Med*. 2007;175:336–44.
8. Cooper PJ, Barreto ML, Rodrigues LC. Human allergy and geo-helminth infections: a review of the literature and a proposed conceptual model to guide the investigation of possible causal associations. *Br Med Bull*. 2006;79–80:203–18.
9. McSorley HJ, Hewitson JP, Maizels RM. Immunomodulation by helminth parasites: defining mechanisms and mediators. *Int J Parasitol*. 2013;43:301–10.
10. Kim SW, Jeon YK, Won TB, Dhong HJ, Min JY, Shim WS, et al. Effects of corticosteroids on expression of interleukin-18 in the airway mucosa of a mouse model of allergic rhinitis. *Ann Otol Rhinol Laryngol*. 2007;116:76–80.

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