

IN VITRO TOXICOLOGICAL ASSESSMENT AND IN VIVO IMMUNOTHERAPEUTIC INVESTIGATION OF RECOMBINANT PROTEINS FROM *Trichuris trichiura*

JESSICA CRISTIANE DA CONCEIÇÃO DE ANDRADE¹; JENNIFER EMILY ANUNCIAÇÃO SOUSA¹; JADE LIZ FERREIRA MENDES SOUZA³; LÓURENCE JOSUÉ W. GOMES¹; CAROLINA ORRICO MELO FERREIRA DE JESUS¹; JOÃO VITOR BORGES RIOS¹; PAULO EMILIO DE OLIVEIRA CRUZ¹; LORENA MIRANDA SOUZA¹; VÍTOR LIMA MIRANDA MELO¹; EMÍLIA MARIA MEDEIROS DE ANDRADE BELITARDO¹; EDUARDO SANTOS DA SILVA¹; CARINA DA SILVA PINHEIRO¹.

¹LABORATORY OF ALLERGOLOGY AND ACAROLGY – LAA; INSTITUTE OF HEALTH SCIENCES, FEDERAL UNIVERSITY OF BAHIA, SALVADOR, BA, BRAZIL; ²GONÇALO MONIZ INSTITUTE, OSWALDO CRUZ FOUNDATION (FIOCRUZ), SALVADOR, BA, BRAZIL

Introduction: Allergic diseases affect millions of people worldwide. Helminths have shown potential in modulating allergic responses in the host. Omics studies conducted by our group identified TtMIF and Ttc4299 proteins as potential modulators of inflammatory responses. **Objective:** To evaluate the cytotoxicity of the recombinant proteins rTtMIF and rTtc4299 from *Trichuris trichiura* in different cell types and rTtc4299 immunomodulatory potential in a murine model of *Blomia tropicalis*-induced allergy. **Methods:** In this study, rTtc4299 was heterologously expressed in *E. coli* BL21 (DE3), purified using an ÄKTA Pure 250 system with a HisTrap FF Ni Sepharose column, and confirmed by SDS-PAGE and Western blot analysis. The cytotoxic effects of recombinant proteins were evaluated on the human macrophage cell line (THP-1) using the MTT assay. The immunomodulatory capacity of rTtc4299 was assessed through cytokine quantification in cell supernatants using ELISA. A/J mice were sensitized and challenged with *Blomia tropicalis* extract, followed by treatment with dexamethasone or rTtc4299 protein via subcutaneous administration. Regulatory T cell production in splenocytes' culture was verified by flow cytometry. **Results:** Stimulation with the proteins did not induce toxicity in THP-1 cells. The rTtc4299 stimuli induced the increased secretion of IL-10, IFN- γ , and IL-1 β levels, while reducing TNF levels, indicating its immunomodulatory properties. Total and differential leukocyte counts in the bronchoalveolar lavage fluid of mice were modulated by rTtc4299 treatment. Antibodies (IgG, IgG2a and IgE) and cytokine responses (IL-10, IL-4, IL-5 and IFN- γ) were also modified in mice treated with rTtc4299. Flow cytometry analyses revealed that rTtc4299 treatment influenced T cell regulatory responses. **Conclusion:** *T. trichiura* recombinant proteins seem to be non-toxic and capable of modulating the Th2 immune response *in vitro* and *in vivo* promoting a mixed immunological profile with regulatory and Th1 cytokine production. However, additional assays are still required to confirm these findings.

Keywords: Allergy, immunomodulation, whipworm

Support: CAPES (88887.935812/2024-00); Fundação Escola Politécnica da Bahia (0156-A); CNPq (403336/2021-0).