Peptides from Amaranth controlled the NF-κB pathway activation on epithelial cells and suppressed intestinal inflammation

Paola L Smaldini, Micaela Quereda, Gastón Rizzo, Maria Lucia Orsini Delgado, Julián Moronta, Cristina Añón, Guillermo Docena

1-Instituto de Estudios Inmunológicos y Fisiopatológicos-IIFP, Facultad de Ciencias Exactas, UNLP. La Plata, ARGENTINA

2-Centro de Investigación y desarrollo en Tecnología de Alimentos (CIDCA), CONICET CCT La Plata y UNLP, Argentina

psmaldini@biol.unlp.edu.ar

Introduction & Aim

Biological, nutritional and health benefits of amaranth have been highlighted in the last years. Proteins of amaranth exert anti-hypertensive, anti-oxidant, anti-thrombotic and anti-proliferative effects. The aim of this study was to analyze the anti-inflammatory effect of peptides from amaranth on NF-kB-intracellular pathway activation in intestinal epithelial cells, and in experimental intestinal inflammation, such as colitis and food allergy.
Previously, we characterized peptides with anti-inflammatory properties in vitro.

Materials & Methods

In vitro assays: immunomodulation of Caco-luciferase cell line
Mouse model: a cholera toxin-driven Th2 specific immune response was promoted in Balb/c mice by gavage, and hypersensitivity reactions were evidenced immediately after the oral challenge with CMP. And a colitis mouse model, Balb/c were intrarectally administrated with TNBS or ethanol (EtOH) at day 0, mice were sacrificed at day 7
In vivo parameters: clinical score, skin test were analyzed, weight
In vitro parameters: serum specific isotypes, cytokines, mucosal Tregs and cytokines, were assessed.
Therapeutic strategies: synthetic peptide of amaranth were orally administrated.

Results

Mouse model of food allergy

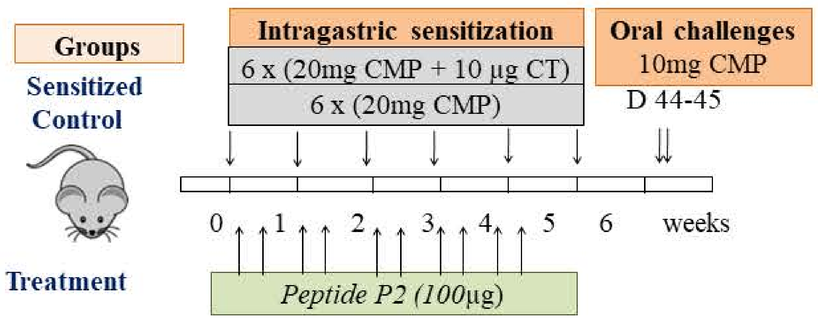


Table 1. Clinical score assigned to symptoms

Score	Symptoms
0	No symptoms
1	Scratching and rubbing around the snout and head
2	Puffiness around the eyes and mouth, pilar erecti, reduced activity with increased respiratory rate
3	Respiratory distress, cyanosis around snout and tail
4	No activity upon stimuli, convulsion
5	Death

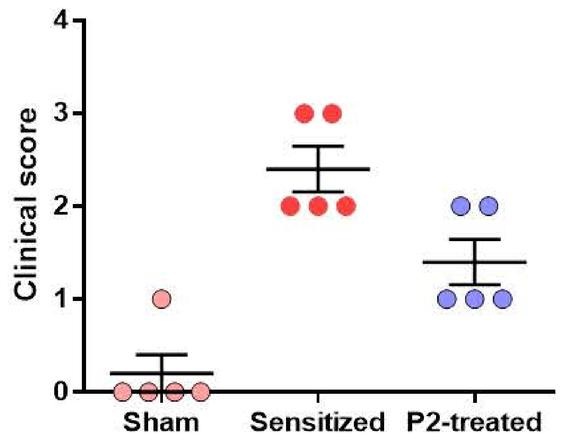


Figure 2. Assessment of the hypersensitivity response: clinical score was lower in treated animals compared with sensitized mice

Figure 1. Sensitization and immunomodulation protocol

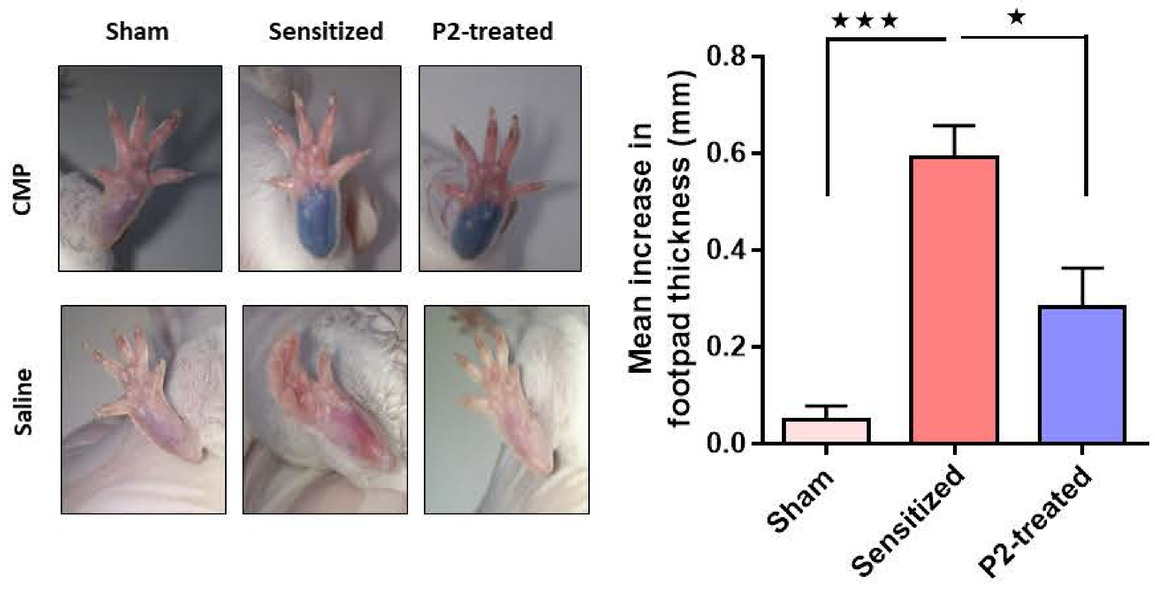


Figure 3. Cutaneous tests after immunomodulation. sensitized animals showed cutaneous inflammation (blue), whereas in treated animals vascular leakage was controlled.

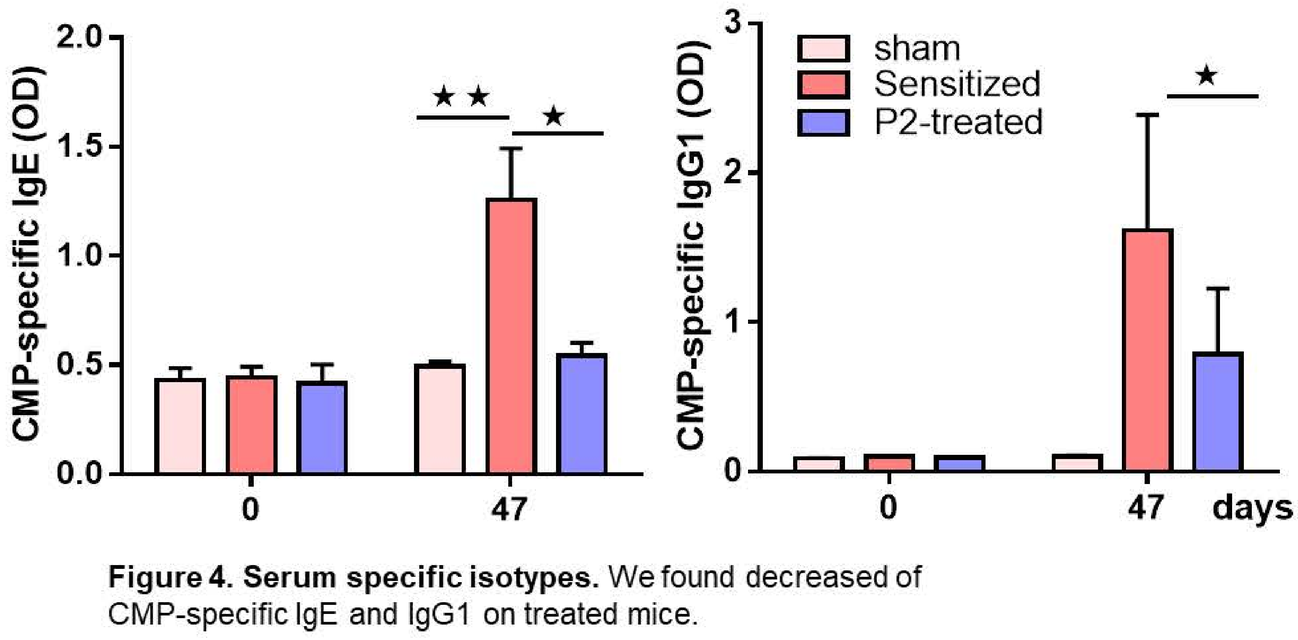


Figure 4. Serum specific isotypes. We found decreased of CMP-specific IgE and IgG1 on treated mice.

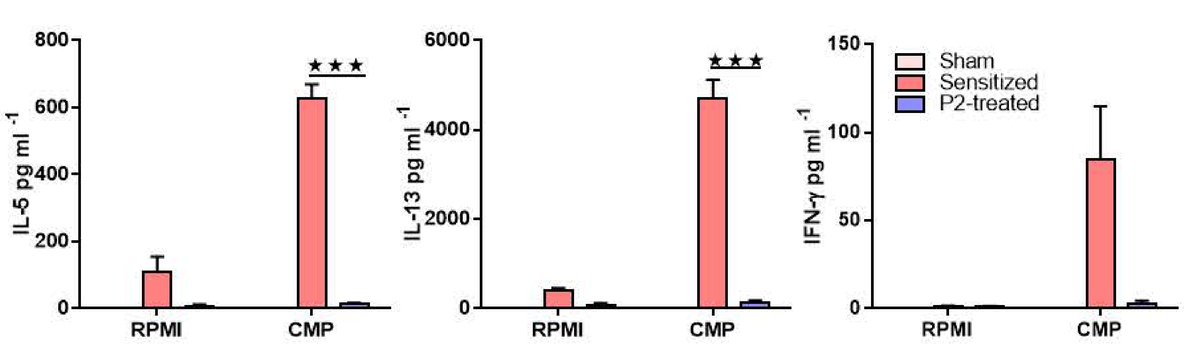


Figure 5. Cytokines. Cytokines in the supernatants of spleen cells stimulated with CMP or medium for 72 h. Treated mice showed lower production of Th2 cytokines.

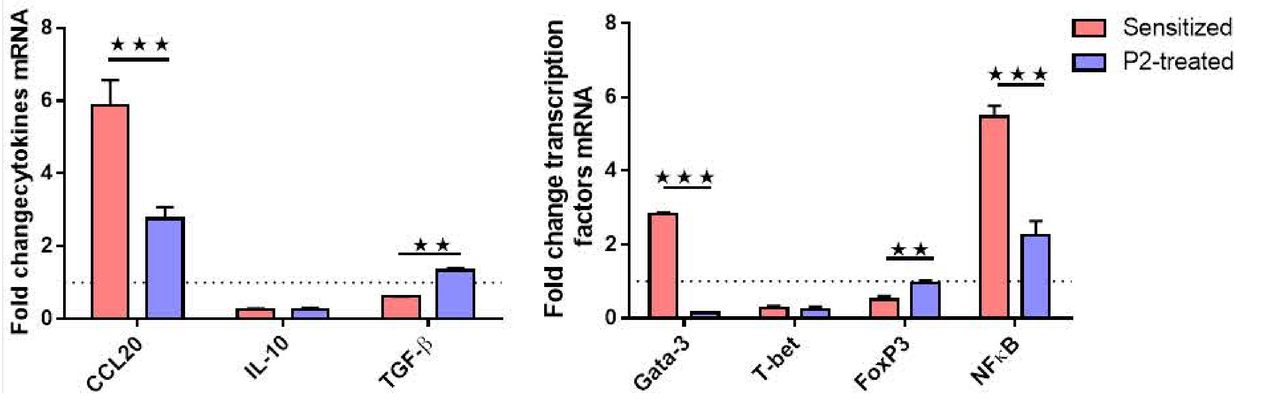


Figure 6. Local cytokine and transcription factors expression on gut: Treated mice expressed less amounts of Ccl20, Gata-3 and NF-κB, with increase in TGF-β and FoxP3.

Mouse model of colitis

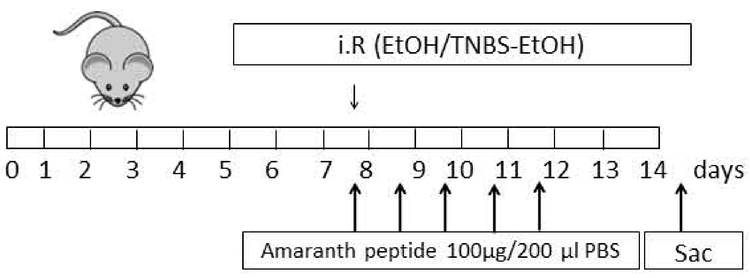


Figure 8. Protocol for induction of colitis.

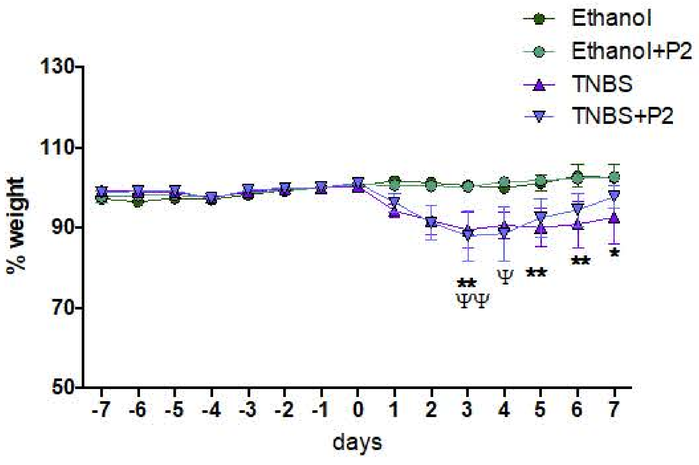


Figure 9. Body weight. Peptide 2 treatment attenuates weight loss in mice with TNBS-induced colitis

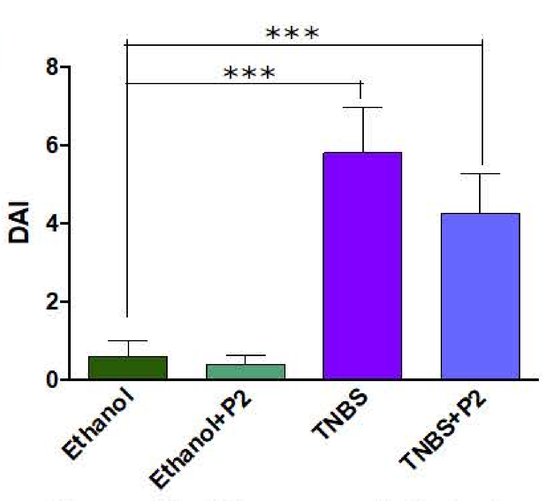


Figure 10. Disease activity index. P2-treated mice showed lower activity.

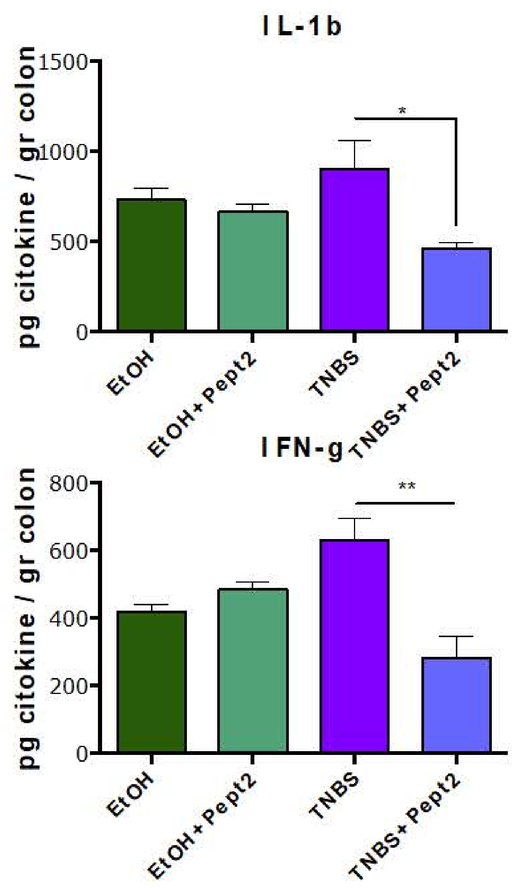
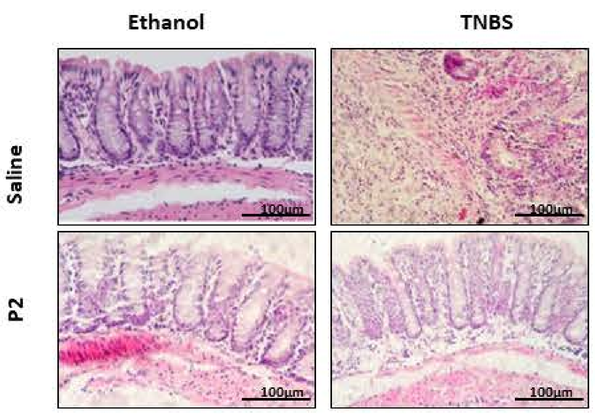


Figure 13. Cytokine production on colon tissue. Mice treated with the peptide produced less amount of pro-inflammatory cytokines



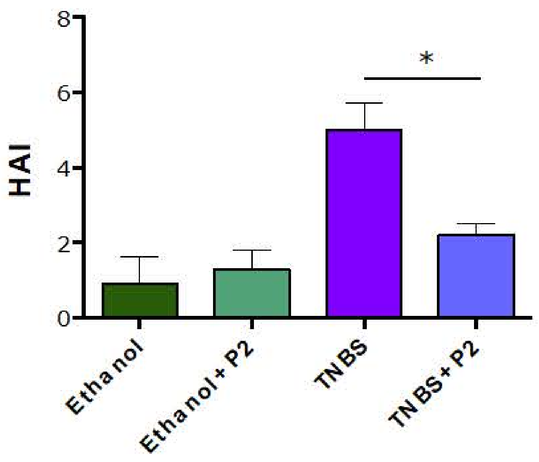


Figure 11. H&E and histologic activity index. P2-treated mice showed a lower recruitment of inflammatory cells. Magnification: 400X, scale bar:100µm

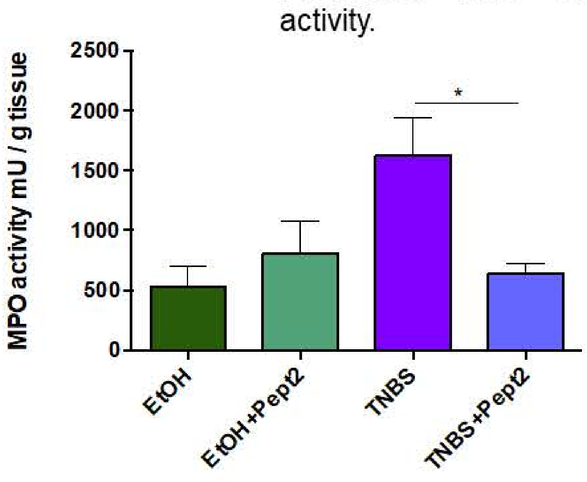


Figure 12. Myeloperoxidase activity on gut.

Conclusions

✓The peptide P2 from Amaranth controlled the Th2-mediated allergic response, decreasing clinical score and cutaneous test *in vivo*, serum IgE levels and Th2 profile cytokines *in vitro*.

✓The peptide P2 from Amaranth ameliorates weight loss, clinical score and mucosal inflammation in a TNBS-induced mouse colitis model.

These findings led us to propose that this peptide might be included in the composition of a functional food