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A shorter an more specific oral sensitization-based experimental model of food allergy in mice

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Background: Cow's milk protein (CMP) allergy is one of the most prevalent human food-borne allergies, particularly in children. The incidence of CMP allergy is increasing and this has been associated with the development of other atopic diseases. Experimental animal models have become critical tools to study the molecular mechanisms involved in this condition and to evaluate new therapeutic approaches. However, oral food allergen sensitization in mice requires long treatments, being usually associated with unspecific immune responses.

Aims: The aim of the present study is to characterize a new food allergy model in mice that takes a shorter period of time (two weeks) than that required in the original protocol (eight weeks). This would constitute an advantage in the evaluation of new pharmacological treatments in food allergy.

Methods: We compare the immunological response of CMP allergy in two protocols developed in female Balb/c mice (3-wk-old, immediately after weaning). 1) In the original protocol, mice were sensitized intragastrically with CMP (1 mg/g mouse) plus cholera toxin (CT) (0.3 μg/g mouse), and boosted seven times in weekly intervals. Seven weeks after the first oral gavage, all groups were challenged intragastrically with a high dose of CMP (35 mg/mouse). 2) In the shorter protocol, mice were also sensitized intragastrically with CMP plus CT at the same doses, but boosted only five times in two weeks. Eighteen days after the first oral gavage, mice were challenged intraperitoneally with 1 mg CMP. Equivalent control groups were used. In both protocols, the hypersensitivity responses were evaluated using a scoring system (0-5). 30 minutes after the last challenge mice were sacrificed and blood collected; then, spleen and intestine segments were removed and weighed. Histamine, immunoglobulins (Igs) (IgG1, IgE or IgG2a) and IL-4 levels were evaluated in the blood and/or in colon homogenates by using commercial kits. Statistical analyses (student's t-test or MannWitney U-test for parametric and non-parametric values) were carried out with Statgraphics 5.0, with statistical significance set at $p<0.05$.

Results: The new shorter protocol has several advantages over conventional protocol (Table 1). In summary, it reduces the experimental time required while maintain the main clinical symptoms of the pathology, namely higher allergy score in comparison with control mice ($p<0.05$) or increased IL-4 plasma levels. Moreover, in the shorter protocol, a more allergen-specific response than that achieved with the original protocol is shown, since a similar increase on the amount of CMP-specific plasma IgG1 is observed in both groups (Table 1) while it does not increase the total amount of Igs in plasma or colon (not shown).

Table 1. Comparison of conventional and shorter experimental model of food allergy

Group	Score (0-5)	Plasma IL-4 levels	Plasma CMP-specific IgG1

(n=10)		**(pg/ml)**	**(arbitrary units)**
Control	0.52 ± 0.23	5.87 ± 0.31	0.27±0.06
Conventional	3.46 ± 0.18*	7.21±1.23*	0.85±0.09*
Short	3.64 ± 0.12*	10.04±0.85*	0.83±0.04*

Data are expressed as mean ± SEM. *p<0.05 vs. Control group.

Conclusion: The new shorter protocol of CMP allergy could become an excellent experimental model for advancing in allergy research and treatment.