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Tolerogenic effect elicited by protein fraction derived from different hypoallergenic formulas in PBMCs from children with cow milk allergy

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Abstract

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BACKGROUND
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are available for the dietary treatment of cow's milk allergy (CMA). Safety and nutritional profile of these formulas have been well evaluated, but the potential tolerogenic activity elicited by their protein fraction is still largely undefined. We aimed to comparatively evaluate the tolerogenic effect elicited by protein fraction derived from different hypoallergenic formulas available for the dietary treatment of CMA

METHODS: Four hypoallergenic formulas were compared: extensively whey formula (EHWF), extensively hydrolyzed casein formula (EHCF), hydrolyzed rice formula (RHF), amino acid based formula (AAF). Formulas were reconstituted in water according to manufacturer's instructions, and subjected to in vitro infant gut simulated digestion using a sequential gastric and duodenal static model. Resulting protein fractions were purified using C18 reversed phase pre-packed cartridges (Sep-Pak, Waters, Milford, MA, USA), recovered in 70% acetonitrile/0.1% trifluoroacetic acid and finally vacuum-dried. Tolerogenic effects were evaluated in peripheral blood mononuclear cells (PBMCs) from 6 patients, with challenge-proven IgE-mediated CMA (age range 1-5 yrs, all Caucasians), stimulated with different doses of digested protein fractions (from 0.25 to 250 µg/ml) or β -lactoglobulin (BLG; 100µg/ml) or bovine serum albumin (BSA; 100µg/ml) as positive and negative control respectively. The production of Th2 (IL-4, IL-5, IL-13) and Th1 (IL-10, IFN- γ) cytokines were assessed by ELISA. Modulatory action was also evaluated on immune (IL-33) and non-immune tolerogenic factors (mucin 5AC, tight-junction proteins ZO-1 and occludin) in human enterocytes (Caco-2 cells) by ELISA and Real Time PCR, respectively.

RESULTS: Th2 cytokines were unaffected by the exposure to protein fraction from all study formulas, whereas only protein fraction from EHCF was able to positively modulate IL-10, IL-33, mucin 5AC, ZO-1 and occludin expression. All protein fraction from study formulas were able to increase INF- γ expression in PBMCs.

CONCLUSION: The results suggest a different regulatory action on immune and non-immune tolerogenic mechanisms elicited by protein fraction from different hypoallergenic formulas.

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