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Effects of several combinations of probiotics and prebiotics on the specific intestinal immune response in ovalbumin sensitized mice

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Introduction: During the last few years many reports about the immunoenhancer capabilities of some prebiotics and probiotics have been published. However, these beneficial effects of probiotics are not only species-specific and strain-specific, but in addition, their immunomodulator capabilities may be enhanced when the bacteria are able to ferment selected prebiotic sources. This may be of particular interest if a probiotic strain isolated from human milk is combined with human milk oligosaccharides, such as Lacto-N-neotetraose (LNnT), as a prebiotic source. Thus, the study of several combinations of probiotic strains with different sources of prebiotics in an immunologic context may yield synergistic combinations with better modulatory capabilities to improve health host.

Objective: The aim of this study was to evaluate the influence of different probiotic strains, in combination with two types of prebiotics, on specific intestinal immune response in intragastrically ovalbumin (OVA)-sensitized mice.

Diets and animals: Two different prebiotics, Synergy 1 (Beneo HP, Orafti) and a prebiotic derived from human milk, LNnT in combination with the probiotic strains *Lactobacillus fermentum CECT5716* (Hereditum® Lc40, Puleva Biotech), *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bl07 (Danisco) were tested. Three experimental diets were used: a standard AIN-93M diet with 5% cellulose (Control diet); the same diet containing 5% Synergy 1 (Beneo) (this prebiotic was provided as a unique source of fibre); and the same diet containing 0.533% LNnT. Probiotics were given at a dose of 1×10^8 cfu/d of each strain in the drinking water. Ninety Balb/c female 6 weeks old mice (Charles River España) were distributed in six experimental groups – one control and five pre-/probiotics combinations ($n = 15$).

Experimental design: Mice started to receive experimental diets and probiotic solutions 7 days before the first intragastric sensitisation with OVA at a dose of 100 µg per mouse. A booster was given 21 days later on. In both sensitisations OVA was dissolved in bicarbonate buffer after an intragastric gavage of trypsin inhibitor in order to avoid loss of epitopes by enzymatic digestion or acid-driven structural changes. Ten days later on, mice were sacrificed and the small intestine and colon were extracted and weighed. Small intestine Peyer's patches were identified and counted, and the intestinal washes from both intestine segments were collected and stored frozen until specific antibodies against OVA were measured by ELISA.

Results: Some of the experimental synbiotics were able to modulate the small intestine weight, although the number of Peyer's patches per intestine was not affected by any experimental combination. Despite no significant effect of any treatment on the total protein amount in both small intestine and colon washes, several combinations of pre- and probiotics were able to effectively modulate the antigen driven antibody response, at the small intestine as well as colon levels. Regarding the small intestine, there was a dramatic fall of about 50% in the amount of immunoglobulins against the antigen in the intestinal lumen induced by the supplementation with the mix of strains *L. acidophilus* plus *B. lactis*, either combined with Synergy 1 or LnNT ($P<0.05$). Likewise, the level of OVA-specific antibodies located at the colon lumen was negatively affected by the group fed LNnT and the mix of *L. fermentum* plus *B. lactis*, although the other three experimental synbiotics seemed also to dampen this response ($P<0.05$).

Conclusions: The three experimental synbiotic groups (the two groups fed the mix *L. acidophilus* and *B.* lactis either with Synergy 1 or LNnT, and the group fed the mix of *L. fermentum* and *B. lactis* plus LNnT) were the most effective in decreasing the specific intestinal response against a well-known foodborne antigen, which may be one of the potential mechanisms by which some synbiotics may play a role in preventing food allergy. On the other hand, the same combinations of pre/pro and synbiotic did not dampen the specific response against an antigen given systemically (data not shown).