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Effects of several combinations of probiotics and prebiotics on the specific systemic immune response in an influenza vaccination mouse model

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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 but also depend on certain strains, and in addition to that, 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.

Objective: The aim of this study was to evaluate the influence of different probiotic strains, in combination with two types of prebiotics on specific immune response in a vaccination mouse model.

Diets and animals: Two different prebiotics, Synergy 1 (Beneo HP, Orafti) and a prebiotic derived from human milk, Lacto-N-neotetraose (LNnT) in combination with the probiotic strains *Lactobacillus fermentum CECT5716* (Hereditum® Lc40, Puleva Biotech), *Lactobacillus acidophilus* NCFM and *B. 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) (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 subcutaneous sensitisation with influenza vaccine (Mutagrip) at a dose of 0.45 μg haemagglutinin per mouse. A booster was given 21 days later on. Nine days after, the ear thickness was measured using a precision electronic caliper before injecting the animals with non-diluted vaccine in both ears. Delayed type hypersensitivity (DTH) response was checked by measuring ear swelling 24 h after the antigen injection. Then mice were sacrificed and the spleen was extracted and weighted. In addition to that, blood samples were collected by puncture of the facial vein on three different days: the days of first sensitisation, booster and once DTH response was measured. Serum-specific antibodies against influenza were measured by ELISA.

Results: Some of the experimental synbiotics were able to modulate some morphohistologic parameters, such as spleen weight. Despite no significant effect of any treatment were found on the influenza-specific serum response, the DTH response was significantly increased by the combination of *L. fermentum* plus Synergy 1 ($P < 0.05$), as well as other combinations which seemed to be effective, although did not reach signification, such as the mix of *L. acidophilus* and *Bifidobacterium lactis* plus LNnT ($P = 0.09$ and $P = 0.11$, respectively).

Conclusions: In summary, three experimental groups might play a role in improving the systemic immune response: the group fed on the combination of *L. fermentum* plus Synergy 1, was the most compelling, followed by the ones given the mix of *L. acidophilus* and *B. lactis* specially combined with LNnT, but also with Synergy 1. However, none of the experimental combinations was able to influence the specific antibody response in serum. The increased DTH responses after vaccination suggest that this dietary supplementation with synbiotics might enhance parameters of Th1-dependent cellular immunity.