

Probiotics and immune regulation : effects of *Lactobacillus casei*

Raphaëlle Bourdet-Sicard

Full abstract :

Probiotics, including Lactobacilli, have been postulated to alleviate allergic and inflammatory diseases in humans. It has been shown that consumption of a mixture of probiotics (called VSL#3) prevent pouchitis. Other *Lactobacillus* or *Bifidobacterium* strains have been used in ulcerative colitis or Crohn's patients. The consumption of several but not all probiotics seems to be a promising way to reduce inflammatory pathologies.

Lactobacilli have been shown to reduce by half the incidence of atopic eczema in young children when administered early after birth.

Nevertheless, mechanisms by which probiotics exert their actions remain poorly understood. To examine whether anti-inflammatory effect of probiotics could involved immune modulation of effector and regulatory T cells, we examine whether *L. casei* DN-114 001 could affect antigen-specific T cell mediated skin inflammation.

Contact sensitivity (CS) to haptens is a type IV delayed type hypersensitivity reaction, mediated by hapten-specific CD8+ T cells, which develop in lymphoid organs within 5 days after epicutaneous sensitization. CD4+ T cells are not necessary as helper cells, but instead regulate the intensity of CS and its resolution. Therefore CS to 2,4-dinitrofluorobenzene (DNFB) appears as a model suitable for investigating the immunomodulatory effect of fermented-milk containing *L. casei* (i.e. Actimel®) and for discriminating the immunomodulatory effect of *L. casei* on CD8+ effectors T cells mediating skin inflammation and CD4+ regulatory T cells.

Mice were sensitized on day 0, with DNFB topically applied onto the shaved abdomen. On day 5 mice were ear challenged with DNFB applied onto the right ear, the left ear received the vehicle alone. Ear thickness was measured with calipers before challenge and each days after challenge. Feeding of mice every day with Actimel® starting from day-14 or from day-21 before skin sensitization until the end of the protocol, resulted in reproducible inhibition of the CS response by 50%. Alternatively, treatment starting from day -7 did not affect the CS response.

Because the CS response to DNFB is mediated by CD8+ effectors, we first examined whether inhibition of the CS response by Actimel® resulted in decreased hapten-specific CD8+ T cell responses. Analysis of the proliferative response of hapten-specific CD8+ T cells, isolated on day 5 after sensitization and restimulated *in vitro* with the hapten, showed that treatment with Actimel® resulted in decrease in hapten-CD8+ T cell proliferation. These

results was correlated with decreased IFN γ production by hapten-specific CD8 $^+$ T cells. Elispot analysis of the frequency of IFN γ -producing hapten-specific CD8 $^+$ T cells showed that Actimel $^{\text{®}}$ reduced the number of IFN γ -producing hapten-specific CD8 $^+$ T cell effectors.

Actimel $^{\text{®}}$ is a fermented milk containing *L. casei* DN-114 001 and the two yoghurt starters, *L. bulgaricus* and *S. thermophilus*. Because *L. casei* DN-114 001 has been previously reported *ex vivo* on Crohn's disease explants and *in vitro* on epithelial cells to have anti-inflammatory properties, we next examined the effect of treatment with live *L. casei* or *L. casei* cell wall on the CS response. Mice treated daily from day –14 with *L. casei* (at a dose similar to that of live bacteria contained in Actimel $^{\text{®}}$), inhibited the CS response by 50%, similarly to treatment with Actimel $^{\text{®}}$. Treatment with *L. casei* cell wall (at a dose similar to that present in the daily intake of Actimel $^{\text{®}}$) resulted in similar reduction in the CS response, suggesting that components of the bacteria cell wall were endowed with the inhibitory effect.

In this model, skin inflammation is regulated negatively by CD4 $^+$ T cells. So, we next determined whether the inhibitory effect of Actimel $^{\text{®}}$ on the CS response was due to an effect requiring CD4 $^+$ T cells. To this end, we tested the effect of treatment in two models of CD4 $^+$ T cells deficiency :

1. C57BL/6 mice depleted of CD4 $^+$ cells by treatment with anti-CD4 mAb
2. MHC class II deficient mice which have a defect of class II restricted CD4 $^+$ T cells due to lack of positive selection in the thymus ($A\beta^{\text{°/°}}$).

Consumption of Actimel $^{\text{®}}$ (from day –14) inhibited the CS response by roughly 50% in control group of C57BL/6 mice, as demonstrated in all previous experiments. Interestingly, treatment did not affect the CS response in anti-CD4-depleted mice and exert only a marginal inhibition of CS in MHC class II-deficient mice. This indicated that the mechanism of action of Actimel $^{\text{®}}$ on CS required MHC class II –restricted CD4 $^+$ T cells and possibly NKT cells.

We have shown that *L. casei* fermented milk was able to reduce hapten-induced skin inflammation in mice. In order to know whether this observation could be extrapolated in humans, a randomized, double-binded placebo-controlled study was conducted on Nickel-sensitized women.

Nickel, as DNFB, induces hapten-specific CD8 $^+$ T cell-mediated skin inflammation. Nickel is found in jewelry, cans,....

A patch containing 5 % Nickel was applied before (patch 1) and after (patch 2) 28 days of Actimel $^{\text{®}}$ or placebo consumption. The intensity of the allergic reaction was monitored by the ICDRG score at 5 readings (24 h, 48 h, 72 h, 96 h, 120 h). 64 subjects were selected and

randomised for inclusion in the study. 61 subjects took part in the entire study (Intention To Treat population) and 56 subjects completed the study without major deviation (Per Protocol population).

There was no significant difference between subjects allocated to group placebo versus those allocated to group Actimel® with regard to age, weight, height and BMI. No problem of compliance has been reported throughout the entire study course. The average compliance level was 99.5% for placebo group and 98.4% for Actimel® group, with no statistical significant difference between each study group.

The main criteria of the study was based on the ICDRG score at the 5 readings of each patch (maximal value and Aire under curve). The difference between the score patch 1 and patch 2 determined if the subject was improved, unchanged or aggravated.

There was no difference for the maximal value of the ICDRG score observed among 5 readings of each patch between the two groups. Interestingly, the score of the first reading, 24 hours after the application of the patch, showed an improvement favourable to Actimel®: 16 (57.1%) of 28 subjects that consume Actimel® improved versus 8 (28.6%) of 28 with Placebo. The difference was statistically significant for the PP population (chi2 p-value=0.031).

The further readings did not show any difference.

Subjective criteria, itchy and burning feeling, were also recorded. There was a trend for Actimel® effect on the itchy feeling in the PP population at the first reading and for the maximal value of the score : 18 (64.3%) of 28 Actimel® subjects improved versus 11 (39.3%) of 28 Placebo subjects (chi2 p=0.061).

There was a significant difference for the burning feeling in Actimel® group versus placebo on the maximal value ICDRG: 16 (57.1%) of 28 Actimel® subjects improved versus 7 (25.0%) of 28 subjects with Placebo (chi2 p= 0.014).

These results show an early effect of Actimel® on the Ni-induced delayed hypersensitivity 24 h after the contact with the allergen. Interestingly, the difference in ICDRG score, the objective effect, is not maintained beyond 24h whereas the subjective effects (burning feeling and itchy feeling) are maintained.

The model used in this study is a patch test and 5% Ni is applied onto the skin of allergic volunteers. This test maximizes the delayed hypersensitivity reaction and has been used because it enables reproducible clinical responses. The transiency of the observed effect of Actimel® is most probably due to the maximizing character of the test has been used: the intensity of the immune reaction probably hides the probiotic effect , which can still be observed on the functional signs.

This kind of discrepancy between objective and subjective criteria has already been observed in other studies involving inflammatory reactions and probiotics.

All together, these *in vivo* and humans results open promising avenues for the use of probiotics in human health.