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Role of Probiotics and Functional Foods in Health: Gut Immune Stimulation by Two Probiotic Strains and a Potential Probiotic Yoghurt

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Abstract: There are numerous reports that show the benefits on the health attributed to the probiotic consumptions. Most of the studies were performed using animal models and only some of them were validated in controlled human trials. The present review is divided in two sections. In the first section we describe how the probiotic microorganisms can interact with the intestinal epithelial cells that are the first line of cell in the mucosal site, focusing in the studies of two probiotic strains: *Lactobacillus casei* DN-114001 (actually *Lactobacillus paracasei* CNCMI-1518) and *Lactobacillus casei* CRL 431. Then we describe same beneficial effects attributed to probiotic administration and the administration of fermented milks containing these microorganisms or potential probiotic yoghurt, principally on the immune system and on the intestinal barrier in different experimental mouse models like enteropathogenic infection, malnutrition, cancer and intestinal inflammation.

Keywords: Mucosal immunity, probiotic, probiotic claims, probiotic fermented milk, systemic immunity.

INTRODUCTION

The intestinal microbiota is composed of many beneficial microorganisms among which lactic acid bacteria (LAB) and bifidobacteria are the most commonly evaluated.

Diet, drugs, chemotherapy, stress, can affect the equilibrium of the intestinal microbiota, and probiotic supplements can repair these deficiencies [1, 2]. Fermented milks and yoghurts containing probiotic microorganisms are widely accepted by consumers.

FAO/WHO defined probiotic as live microorganisms which, when administered in adequate amounts confer a health benefit on the host [3]. The beneficial claims for probiotic or fermented milk consumption are numerous and include: increase in the resistance to pathogens, prevention of cancer [4-6], regulation of peristalsis, diminution of the symptoms of lactose intolerance [7], decrease in gut inflammatory response and prevention of food allergy [8]. It was demonstrated that the immune system plays an important role in most of the benefits associated to probiotics.

Considering that fermented products containing probiotics enter to the host by oral way, the gut is the first site where they exert effect, and then, by the common mucosal immune system, they can affect other distant organs and also the systemic immunity. The knowledge of how these microorganisms can affect the systemic and mucosal immunity is very important to found the scientific basis for the use of probiotics or fermented milks and to determine how these non-pathogenic microorganisms included in the daily diet of many people can influence the host's immunity.

The use of animal models is very important to understand, in a controlled environmental, the modulation of the host's immune response that is an important effect associated to the benefits observed with most probiotics. In this sense, the importance of understanding the immuno-modulatory capacity of probiotics was analysed [9]. Recently, the immune system stimulation by different probiotic microorganisms was reviewed [10]. Probiotics can improve non-specific immune response by activation of macrophages, natural killer (NK) cells, cytotoxic T-lymphocytes. Different probiotic microorganisms can induce different cytokine responses, and the increased number of IgA secreting cells was associated to the improvement of gut mucosal immune system by probiotics and fermented products containing these microorganisms. The importance of immunomodulation by probiotic was also related to the normalization of dysbiotic microbiota associated with immunopathologies [11]. However, if we refer to specific diseases, there are not enough human trials where the application of probiotics as biotherapeutic agents was evaluated in double-blinded large scale clinical trials. These assays are very important before the medical community accepts the addition of probiotic as supplements for specific patients. This review summarizes some of the most relevant results reported for two probiotic strains, *Lactobacillus* (*L.*) *casei* CRL-431 and *L. casei* DN-114001 (actually *L. paracasei* CNCMI-1518), in healthy host and also in animal models for specific pathologies. The

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effect of yoghurt composed by potential probiotic strains will be also described in a model on inflammatory bowel disease. In all the models, the relation of probiotic consumption with the host's immune system will be discussed.

CONTACT OF *Lactobacillus casei* DN-114001 WITH GUT EPITHELIAL AND IMMUNE CELLS

The abilities to persist in the intestine and to adhere to this surface are important characteristics utilized to select probiotic microorganisms [12].

The intestinal epithelial cells (IECs) are the first cells that interact with probiotics at the intestinal level. Increased adhesion of the microorganisms can enhance their survival in the gastrointestinal tract, helps to counter the effect of the peristalsis and provides a best activation of the IECs.

The contact of the probiotic microorganism with the IECs is the first stage needed for the transduction of signals that stimulate the immune response in the intestine and this fact was demonstrated for *L. casei* DN-114001 by electron microscopy using a mouse model (Fig. **1**). Mice were orally given a suspension of *L. casei* DN-114001 (10^9 CFU/ml), and the samples from the small intestine were obtained at 10 min and used for microscopic observations. It was observed the interaction of *L.casei* DN-114001 with the enterocytes (microvilli, Fig. **1**), similar to the results obtained previously for the probiotic strain *L. casei* CRL 431 [13], and this interaction activated the IECs by increasing the multivesicular bodies in their cytoplasm [14].

Toll like receptors (TLR) can be expressed in the IECs and the stimulation of these receptors allows them to produce cytokines such as IL-6, IL-1 and IL-8 [15]. It was reported that the co-culture of a cell suspension enriched in IECs with the probiotic bacterium *L. casei* CRL 431 activated these cells stimulating the release of cytokine [16]. The *in vivo* activation of the IECs by the probiotic fermented milk (PFM) that contained the starter cultures of *L. delbrueckii* subsp. *bulgaricus* (10^8 CFU/ml), *Streptococcus thermophilus* (10^8 CFU/ml) and the probiotic bacterium *L. casei* DN-14001 (10^8 CFU/ml) was evaluated through the measurement of IL-6 release by IECs obtained from mice given PFM [14]. It was reported that mice given PFM significantly enhanced IL-6 production by the IECs being these increases lower than the induced by a pathogenic bacterium [17]. This cytokine is important to initiate and maintain the cross-talk of the IECs with the immune cells in the intestine, and it was also reported that is involved in the induction of immunoglobulin A (IgA) B cell clonal expansion [18]. The interaction of *L. casei* DN-114001 with the gut associated immune cells was also examined in a mouse model using bacteria labelled with fluorescein isothiocyanate (FITC). The results showed that the whole bacteria or their fragments interacted with the cells of the immune system (fluorescent cells) located in the Peyer's patches (PP) and in the villi (lamina propria). After 10 min of probiotic administration the fluorescent cells were observed in PP and in the lamina propria, they reached a peak at 24 h, and gradually diminished until 72 h [14]. The decrease observed in the number of fluorescent cells means that the whole bacterium or the bacterial fractions did not colonize for a long time in the intestine, remained a short time and then continued the normal clearance. This is important to consider that this bacterium is introduced in fermented milk that is consumed on a regular diet by the hosts.

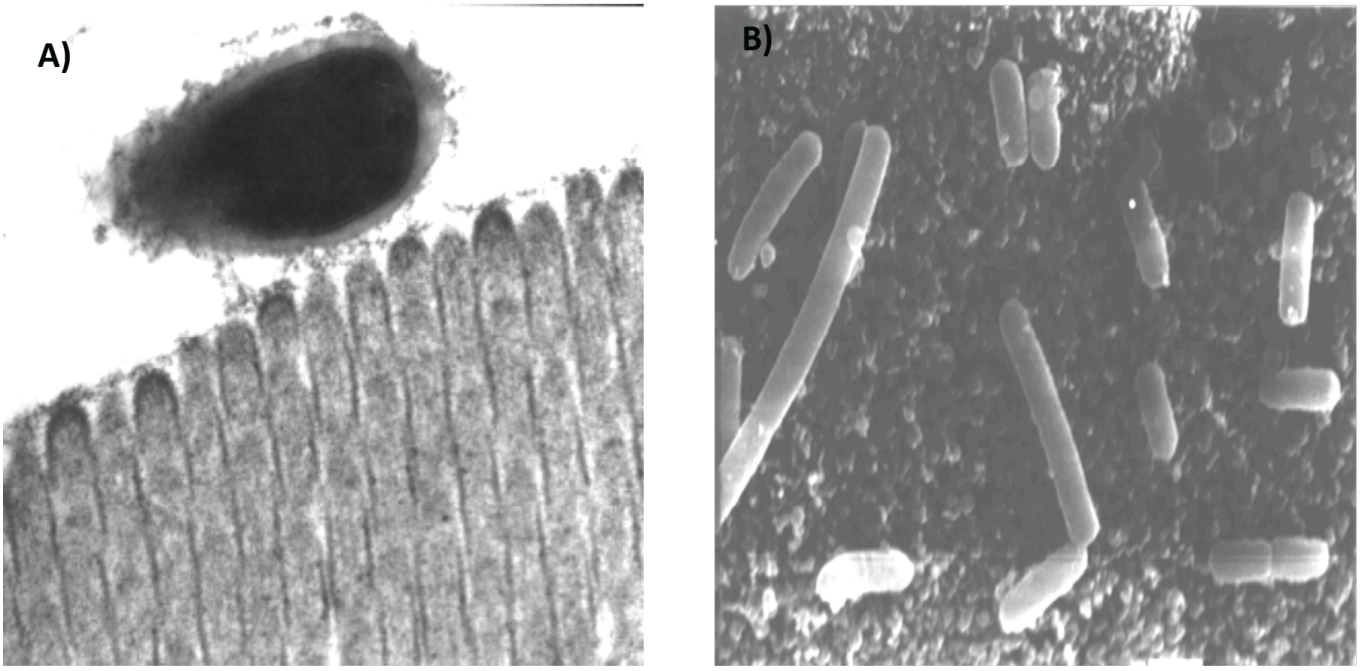

Fig. (1). Adhesion of *L. casei* DN-114001 to the epithelial cell. Representative microphotographies obtained with transmission electronic microscopy from samples of the small intestine of a mouse given *L. casei* DN-114001. **A**) Transmission electronic microscopy of *L. casei* DN-114001 adhered to microvilli in the apical surface of epithelial cells. **B**) Scanning microscopy of the epithelial cell and the *L. casei* DN-114001.

These results also agree with the ones obtained with *L. casei* CRL 431 [13], where it was suggested that fluorescent cells detected after the administration of FITC-labelled bacteria to mice were immune cells that interacted with bacterial fragments. However, the presence of microfold cells (M cells) in the intestinal villous possibilities that the whole bacteria could also have translocated to the lamina propria through these cells [19] or by the dendritic cells (DCs), that express proteins like ones present in the tight junctions, allowing them to extend cell prolongations between the intestinal epithelial cells, without affect the intestinal permeability [20].

PROBIOTIC FERMENTED MILK ADMINISTRATION AFFECTS INTESTINAL NON-ESPECIFIC BARRIER AND IMMUNE CELLS

A protective mucus layer covers the epithelium of the gastrointestinal tract. This layer functions as an active defensive barrier that is synthesized and secreted by goblet cells and that increase in response to gut microorganisms [21]. It was observed that mice given PFM containing *L. casei* DN-114001 enhanced significantly the count of goblet cells (Fig. **2**) compared to the animals without special feeding [14].

Probiotic bacterium or bacterial fragments can make contact with the intestinal immune cells as was expressed above. This stimulation can induce modification in the number and function of certain immune cells. It was reported for the probiotic *L. casei* CRL 431 that when it was administered to healthy mice enhanced the count of IgA+ cells and cells associated to the innate immunity, but CD4+ or CD8+ T lymphocytes did not modify in the small intestine [22]. Enhanced intestinal IgA production is an effect desirable with probiotic administration because constitutes a mechanism of defence for the mucosal surfaces [23]. At difference, for the PFM containing *L. casei* DN-114001, it was reported that the administration to mice during 98 days increased not only IgA+ cells but also increased significantly CD8+ and CD4+ cells in samples of the small intestine [24]. This result shows that benefits induced by each probiotic are strain dependent. It is important to consider that the PFM as whole product, contain other compounds such as bioactive peptides, produced during the fermentation process and they could be related with these additional effects.

It was demonstrated that gut innate immune response is stimulated by probiotic administration [25]. Macrophages are essential in the innate immunity and they also participate in the acquired immunity [26]. DCs and effector molecules produced by them (cytokines and chemokines) play also an important role in the innate immune response and in the initiation of the adaptive immunity [27]. The effects of PFM containing *L. casei* DN-114001 on macrophages and DCs were reported by Maldonado Galdeano *et al.* [14]. It was described that macrophages (F4/80+ cells) were significantly enhanced in the lamina propria of the small intestine from mice given PFM compared to animals without especial feeding; however PFM administration did not affect the number of DCs. It was also reported that these changes in the number of macrophages were not associated to modifications in the expression of cell receptors, such as CD 206 and TLR-4, which biological signals are implicated in their activation [14].

Calcineurine (CN) was also analysed because it was reported that this molecule participates in the coordinated stimulation of the Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) and IL-2 genes, mediated by the transcriptional nuclear factor of activated T-cells (NF-AT) in T lymphocytes [28]. This enzyme has been described in macrophages [29], so CN might be also associated to the stimulation of both macrophages and T lymphocytes. In this sense, it was reported that mice given PFM increased the count of CN+ cells in the lamina propria of their small intestines [14].

Cells that secrete cytokines in the intestine were examined in a mouse model of prolonged time (98 days) continuous consumption of fermented milk that contained the probiotic bacterium *L. casei* DN-114001.

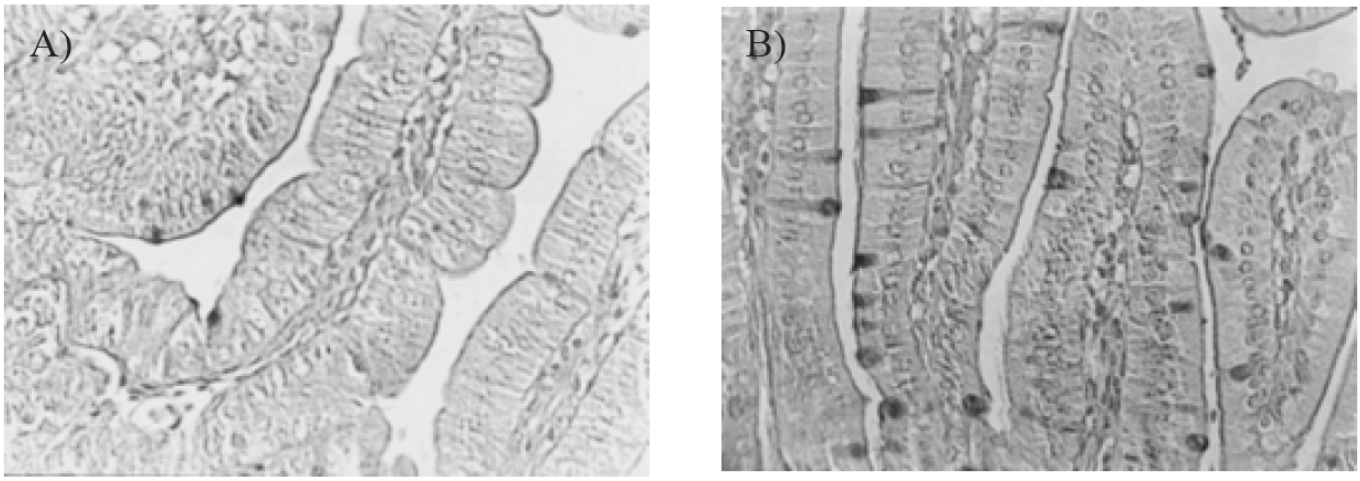

Fig. (2). Effects of PFM administration on the goblet cells. Goblet cells (dark cells) were studied in the lamina propria of the small intestine of mice that received PFM during 5 days. **A**) Representative microphotography from a control mouse without especial feeding. **B**) Representative microphotography from a mouse given PFM during 5 days.

IL-2 is a cytokine that acts as a growth factor for T lymphocytes [30]. It is produced by these lymphocytes and also by DCs [31]. The enhancement observed for IL-2 (+) cells in the mice given PFM agrees with the enhanced count of T cells observed in the intestine of these animals. Tumour necrosis factor alpha (TNFα) is a cytokine produced by stimulated macrophages/monocytes, fibroblasts, mast cells, and also by some T and natural killer (NK) cells. Interferon gamma (IFNγ) is produced by activated T cells, NK, macrophages and DCs [32]. These both cytokines are produced by activated cells and activate other cells that participate in the inflammatory responses and they are known as pro-inflammatory cytokines. However, it was reported that participate also in the crosstalk of the immune cells [33], and the production of these cytokines was increased in the intestine of mice given other probiotic bacterium [13]. In agreement to these results, in the model of mice that received PFM, the number of cells secreting both TNFα and IFNγ enhanced in the intestines. However, no inflammation was observed in these animals, suggesting that these cytokines could affect other process in the gastrointestinal tract (i.e. apoptosis for TNFα) or that the pro-inflammatory effect was regulated. The increases observed for IL-10+ cells agree with this last possibility [24]. IL-10 is a cytokine with regulatory properties that have a key role in the intestine and its deficiency is related to inflammation [34].

EFFECT OF CONTINUOUS PROBIOTIC FERMENTED MILK ADMINISTRATION ON IMMUNE CELLS OUTSIDE THE INTESTINE

Continuous consumption of PFM that contain *L. casei* DN-114001 during long time (98 days) was also evaluated outside the intestine. It was reported that macrophages isolated from the peritoneum of these mice increased their phagocytic capacity only in the beginning of the experiment (until 14 days) [24]. This long term continuous PFM administration was also able to activate the IgA cycle, and the count of IgA producing cells increased not only in the small and large intestine, but also in other mucosal sites outside the intestine such as bronchus and mammary glands. It is important to note that unlike the results obtained in the intestine, the increases of IgA secreting cells in the sites distant from the intestine were only observed in the first samples. It was also described that the counts of CD4+ and CD8+ T lymphocytes did not modify after PFM administration in these mucosal sites during all the experiment. These results agree with the theory of mucosal compartmentalization and the needed of local stimuli to induce the migration of the cells from gut to other mucosal sites [35]. Similar results were obtained in mice given the probiotic *L. casei* CRL 431 which administration was assayed in short periods of time [23], and agree with other study where after the injection of tumour cells in the mammary glands (local stimuli), mice given *L. helveticus* R389 showed a significant enhance of IgA+ cells and T lymphocytes in the mammary glands [36].

According to these results, and at difference of the results observed in the gut, the cytokine producing cells did not modify in both bronchus and mammary glands of mice given PFM compared to those without especial feeding [24].

PREVENTIVE AND THERAPEUTIC EFFECTS OF MILK CONTAINING A PROBIOTIC BACTERIUM IN AN ENTEROPATHOGENIC INFECTION MODEL

Enteric infections caused by bacteria are an important medical problem in development and under development countries. They are the major cause of mortality in children in these countries, and also are a risk for the travellers. It is also important to consider that antibiotics should be used carefully in public health because of the complications associated to it (drug-resistance, chronic toxicity). Actually no oral vaccines are available to protect against diarrhoeas of different aetiologies. Thus the prevention of these diseases by oral administration of probiotic bacteria having an immunomodulatory effect is an attractive possibility. Many mechanisms were proposed for the effectiveness of probiotics in the protection or as therapy against enteropathogens, among them the competition for nutrients, the production of inhibitory components or antimicrobial agents that act against pathogens, the modulation of toxin production and the modulation of host's immune response [37-39]. Considering the immunomodulatory capacity of PFM containing *L. casei* DN-114001, the effect of this probiotc product was evaluated in mouse models of enteric infections caused by enteroinvasive *Escherichia coli* or *Salmonella enterica* serovar Typhimurium.

The model of *E. coli* infection, in which the animals were fed preventively for 5 consecutive days with PFM diluted to a concentration of viable *L. casei* DN-114001 of 10^7 CFU/ml showed that the colonization of the pathogen in the liver and spleen was less compared to control mice that did not received any special feeding. These results were associated to increased levels of anti-*E. coli* secretory IgA in the fluids obtained from the intestine of mice given PFM [40].

Another experiment reported that in mice infected with *Salmonella* the administration of PFM containing *L. casei* DN-114 001 after *Salmonella* infection diminished the severity of the infection; and the benefits of continuous PFM administration (before and after infection) was also demonstrated [41]. Mortality rate and the spread of the pathogen to organs decreased in mice that received PFM. The analysis of the small intestines obtained from these mice showed increased counts of IgA secreting cells in the tissues and total secretory IgA (s-IgA) in the intestinal content. The macrophage inflammatory protein 1α (MIP1-α) was a chemokine also analysed in this model of *Salmonella* infection. MIP1-α is produced by monocyte / macrophages in response to the lipopolysaccharide (LPS) stimulus and acts as a chemoattractant for a variety of cells [42]. Continuous PFM administration induced a fast reaction of the immune response with increases of MIP-1α+ cells since 2 days post infection. These increases of the chemokine MIP-1α were related with a higher afflux of cells from the first line of the immune response such as macrophages. In this sense, it was reported that microbicidal activity of these phagocytes (obtained from Peyer's patches) increased the day of the infection in animals given PFM compared to the control group. Finally, TLR-4 was analysed because is required to control the *Salmonella* infection. The results demonstrated that the count of cells expressing TLR-4 decreased after *S.* Typhimurium infection in all the animals compared with the

data obtained previous to the infection. These decreases were remarkable in the group of animals that received PFM continuously or post-infection. It would be associated to the reduction in the severity of the infection observed in these animals, where, a faster diminution in the count of *Salmonella* in spleen and liver was described compared to the infected animals without any especial feeding. These results can be explained because during *Salmonella* infection, the activation of TLR-4 is related with the production of pro-inflammatory mediators by the cells of the innate immune system, but this response is then followed by the suppression of its own mRNA expression [43].

These results agree with the ones obtained using the probiotic strain *L. casei* CRL431 and demonstrated by Castillo *et al*. [38, 39].

PROBIOTIC FERMENTED MILK ADMINISTRATION EXERTED A BENEFICIAL EFFECT IN AN EXPERIMENTAL NON SEVERE MALNUTRITION MODEL

It is known that malnutrition is the cause of a lack of balance between the nutrient consumption and the energy requirement. The malnutrition not only affects body growth and development but also affects the immune system, being the infections more frequent in malnourished.

The administration of PFM containing *L. casei* DN-114001 as a re-nutrition supplement was analysed in mice under non-severe protein-energy malnutrition. After malnutrition period (loose the 25% of the body weight), mice received different dietary supplements (milk, PFM or the bacterial free supernatant obtained from the PFM, BFS) during the re-nutrition period. It was observed that mice given PFM or its BFS as a supplement of the re-nutrition diet increased the body weight and improved the intestinal histology affected by the malnutrition, faster than the mice given whole milk. The administration of PFM affected also beneficially the intestinal microbiota of the animals with increases of bifidobacteria population [44]. These finding were important because many benefits, especially on the immune system, such as enhanced production of sIgA [45]; stimulation of phagocytosis by mononuclear cells [46]; increased lymphocyte responsiveness to antigen administered orally and systemically [47, 48], were attributed to bifidobacteria.

Different immunological parameters were also evaluated and it was showed that the count of IgA secreting cells, macrophages and DCs increased in the mice given PFM as a re-nutrition supplement, compared to the malnourished control group. These mice not only increased the counts of these cells but also their activity, observed by the increased production of cytokines such as IFN-γ, TNF-α, IL-12. The activation of the immune system in mice that received PFM supplementation during the re-nutrition was also observed with the evaluation of the macrophages obtained from peritoneum and spleen, which increased the percentage of phagocytosis [44].

It was also reported that the malnutrition diminished the systemic immune response against OVA antigen in the mice, and the three diet supplements evaluated during the re-nutrition period improved this response, and no significant differences were observed between them. The effect of PFM administration as a re-nutrition supplement was also analysed in a *S.* Typhimurium infection model. Mice that received PFM before and after infection diminished the translocation of this pathogen to liver; compared to the mice given milk or BFS. This result agrees with the ones obtained previously where, well-nourished mice that received PFM had better protection against *Salmonella* infection, with enhancement of the intestinal and systemic immunity, as was described above.

The effect of this PFM was also examined in the thymus of malnourished mice, after re-nutrition period [49]. It was observed that PFM administration was more effective than the other diet re-nutrition supplements in the improvement of thymus histology. Mice that received this probiotic supplement during the re-nutrition decreased cellular apoptosis, recovered the ratio of CD4 / CD8 single-positive thymocytes, and increased cytokine productions in this organ.

These results showed that the administration of PFM as a diet supplement for the re-nutrition of malnourished mice improved the intestinal immunity and exerted a protective effect against *Salmonella* infection, and served also as adjuvant of the systemic immunity, as was also previously demonstrated using yogurt supplement in a severe malnutrition model [50].

EFFECT OF PFM ADMINISTRATION TO MOTHERS DURING NURSING OR TO THE OFFSPRING AFTER WEANING

The analysis of the administration of PFM containing *L. casei* DN-114001 in early periods of the life was reported using a mouse model. It was demonstrated that the consumption of PFM, either by the mothers during suckling period or by their babies after weaning, increased the counts of bifidobacteria and decreased enterobacteria in the samples obtained from the large intestine of the new-borns. This effect is desirable because many benefits have been attributed to bifidobacteria [51-53]. In that study, the analysis of IgA secreting cells in samples from the small intestines of new-born mice showed that the consumption of PFM by their mothers during the nursing did not influence the counts of these cells, compared to the control group whose mother did not receive any especial feeding. It was also reported that after weaning, the increase of IgA secreting cells was progressive in the new-borns from the control group, without especial feeding. This finding was related to the maturity of the own adaptive immune system in the babies. In contrast, it was observed a lower count of IgA secreting cells at day 28 in mice from mothers that received PFM during suckling period, and it was suggested that this last finding was the result of the protective effect exerted by the passive immunity acquired through breast feeding, which has been reinforced by PFM consumption by their mothers. At 45 days of life, when mice were matured from the immunological point of view, the effect of the administration of PFM to the mothers was not evidenced, and the values were similar to those obtained in the animals from mothers that never consumed the PFM.

The effect of the PFM administration was also analysed on the s-IgA concentrations in the intestinal fluids. It was reported that mice from mothers that received PFM increased s-IgA concentration in their small intestines at age of 12 days. This finding was related to the increase of IgA in the breast milk of mothers that received PFM. It was suggested that antibodies from the mother could exert suppression on the progress of mucosal immunity in their babies, and in this conditions, the immune system of the offspring is partially developed at weaning [54].

Macrophages and DCs were also evaluated in the small intestine of the offspring because, as was explained above, they are key cells of the innate immunity, and also participate in the initiation of the adaptive immunity. Intestinal DCs were expected to regulate the immune response to the intestinal microbiota. Hart *et al*. evaluated different probiotic bacteria and reported that they had difference in their immunomodulatory properties, and affect the polarity of the immune responses by DCs [55]. In this sense, bifidobacteria strains showed remarkable anti-inflammatory properties which were associated to increased production of IL-10 by DCs [54]. In the model of mothers and offspring given PFM, the administration of this probiotic product to the mothers down regulated both macrophages and DCs in the small intestine of their babies on day 12. It was suggested that the mothers protected their offspring with the passive immunity or that the different microbiota influenced the down regulation of these cells at this time point. An equilibrated and complete bacterial colonization was favoured with this situation because an increased activity of these immune cells (participating in the phagocytic activity and the antigen presentation) would not be beneficial for the bacterial colonization. It is remarkable to note that new-born mice from the group that never received PFM did not show this immunoregulatory effect.

It was also observed in mice that reached their immunological maturity, with a complete microbiota establishment in their intestine, that the consumption of PFM by them increased the count of macrophages and DCs. These results are in agreement with other previous observations that were also referred above in this review, where the administration of PFM to adult mice stimulated their mucosal immunity with increased production of different cytokines by T cells, macrophages and DCs [56].

Finally, the analysis of mucus producing cells showed that their numbers decreased in the first sample (12 days of age) in the mice from mothers given PFM during suckling period; after which the number of goblet cells reached, and maintained during all the experiment, values similar to the control (independently of the PFM administration after weaning). After weaning, the administration of PFM to the offspring affected the number of goblet cells only in the babies whose mothers never were given PFM. These results suggest again the influence of the passive immunity provide by mothers, which was reinforced for the administration of PFM as was demonstrated for IgA secreting cells.

YOGHURT IN THE PREVENTION OF INTESTINAL INFLAMMATION AND COLORECTAL CANCER

The effects of probiotics and fermented products on intestinal diseases have been extensively examined considering that these microorganisms enter the organism orally and can positively modulate the intestinal microbiota involved in many of these disorders. It has been shown that LAB and other probiotic microorganisms can counteract inflammatory bowel diseases (IBD) by equilibrating the intestinal microbiota, stabilizing the intestinal barrier, and by altering the immunogenicity of enteral antigens enhancing their degradation [57].

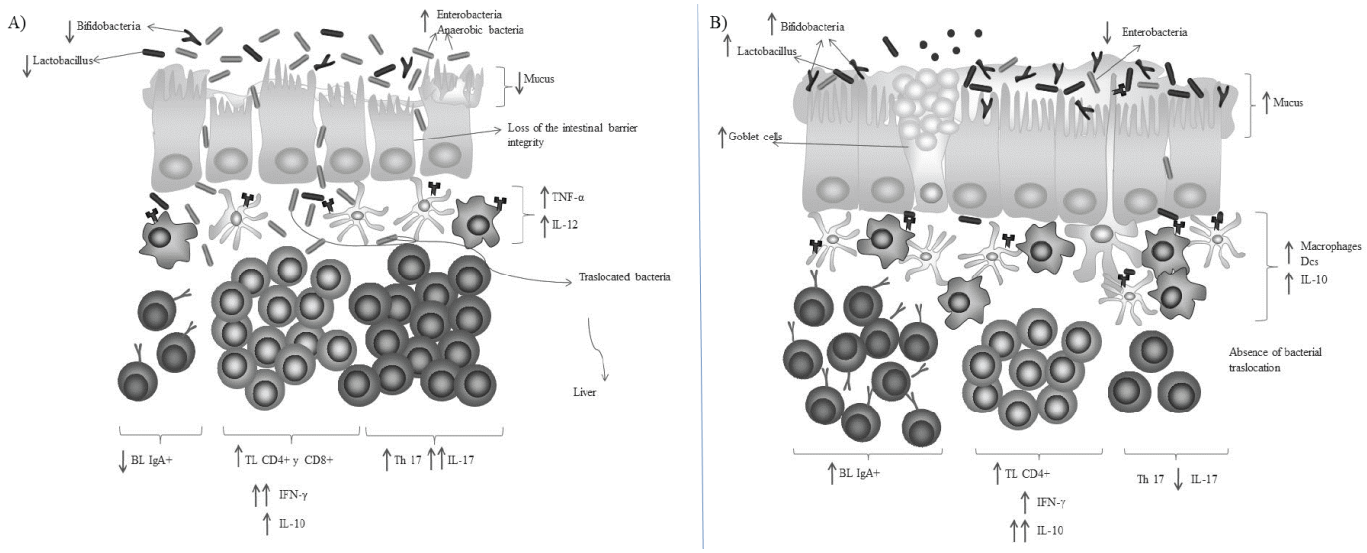

Fig. (3). Effects of yoghurt administration in a TNBS-induced colitis model. Figure A represents the most important modifications observed in the intestine of mice inoculated with TNBS. They present an imbalance of the gut microbiota and typical inflammatory response with increases of Th-17 and CD4+ and CD8+ T lymphocytes. Figure B shows the improvements observed in mice that received yoghurt. They increased bifidobacteria and lactobacillus, mucus producer cells, and showed an anti-inflammatory profile with decrease of Th-17 and CD8+ T lymphocytes, increase of IL-10+ cells, IgA secreting cells, macrophages and dendritic cells (DCs).

It was demonstrated, using an acute trinitrobenzene sulfonic (TNBS)-induced mouse model of IBD, that the administration of yoghurt with potential probiotic strains, decreased the inflammation by modulating of the host immune response [58]. The same yoghurt administered during the remission period in a chronic TNBS- induced model of IBD, prevented the recurrence of the inflammation in these animals maintaining an anti- inflammatory profile of cytokines in the intestine [59]. These both effects were related to beneficial changes in the microbiota of the mice, associated to increases of bifidobacteria population. Fig. **3** summarizes the results obtained in this experimental model.

This anti-inflammatory effect observed in mice that consumed yoghurt was also associated to the anti-tumour potential of this fermented product. Yoghurt feeding inhibited tumour growth in a DMH-induced colon cancer model in mice by modulating of the host's immunity [60, 61]. The analysis of cytokine producing cells showed that yogurt administration stimulated cytokine production in the intestine when this was required; but maintained a regulated immune response. IL-10 was suggested as a cytokine involved in this regulatory response because its production was increased in the intestine of mice that received yoghurt [62, 63]. The analysis of the activity of pro-carcinogenic enzymes demonstrated that mice fed cyclically with yoghurt decreased activity of these enzymes in the intestinal content compared to the tumour control group, in which the increased activity of these microbial enzymes would contribute to the cancer development [64]. These results agree with others in which probiotics *L. rhamnosus* GG and *L. acidophilus* suppressed pre-neoplastic aberrant crypt foci in early stage of colon carcinogenesis using a DMH-induced model in Sprague Dawley rats, and this effect was related to decrease of pro-carcinogenic faecal enzymes [65].

CONCLUSIONS

This review shows the beneficial effects of two probiotic strains which were related to their immonomodulatory potential, especially at the intestinal level. There is not a unique mechanism of action by which probiotics can improve the host´s immune system and they are strain dependent. The beneficial effects associated to the immune system stimulation by the administration of fermented milks containing these probiotic microorganisms were also demonstrated in animal models of different pathologies. Finally the benefits associated to probiotic in inflammatory bowel diseases and colon cancer were analysed by showing results obtained with the administration of a potential probiotic yoghurt in animals models of these pathologies. The use of animal models is very useful to understand the mechanisms of action related to each probiotic; however, these effects should be validated in controlled human trials. In this sense, it is important to distinguish probiotic microorganisms that are included in medicines from other that are included in functional foods. Among functional foods, fermented products are the most common as source of probiotics. In this foods, it is important to consider that in addition to the microorganisms, other products are released as consequence of the matrix fermentation (peptides, fatty acids, enzymes, etc), and they can be also involved in the beneficial effects of the fermented products. There are several reports about clinical trials using probiotic microorganisms, and considering that the effects are dependent of each specific strain, studies are needed to demonstrate the benefits of specific probiotic or fermented product. The realization of double-blinded large scale clinical trials are very important before the medical community can accept the addition of probiotic as supplements for specific patients.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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