

IMMUNE SYSTEM

IS IT POSSIBLE TO MODULATE IT
WITH PROBIOTICS?

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IMMUNE SYSTEM AND ENTERIC FUNCTION

In recent years, the scientific interest concerning the positive effects of some nutrients on the immune system has increased.

The importance of a correct diet in order to obtain adequate development and functioning of the immune system is already recognised: in this sense, infancy constitutes a moment of particular interest (particularly breastfeeding and weaning).

Intestinal surface represents an enormous exchanging area with the external environment (in the adult that surface is equal to a tennis court): it's intuitive that at this level the immune system has a crucial role.

The development and the functional mechanisms of the enteric immune system are relatively independent from the systemic immune system¹ and at this level the interaction between microorganisms and nutrients could influence the inborn mechanisms regulating enteric immune function, especially in the first years of life.

Lymphoid tissue associated with the gut is a secondary lymphatic organ able to process the antigens interacting with the enteric mucosa and then to spread immune reactions.

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PUBBLICAZIONE
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REGISTRAZIONE
DEL TRIBUNALE
DI MILANO N. 567
DEL 17.09.1999

Antigens present in the enteric lumen are carried by micro-fold (M) cells to the Peyer's patches, localized within the enterocytes in the intestinal epithelium (Fig.1). Once they've reached the inside, the antigens interact with the APCs (Antigen-Presenting Cells) who present themselves to the immature B and C cells placed in the germinal centres and in the inter-follicular regions. The B and T cells activated by the same antigens are firstly carried to the regional lymph-nodes, afterwards reaching the systemic circulation through the thoracic duct. Finally, they are differentiated in mature effectors cells, migrating in the lamina propria and on the epithelium of the intestine. For this reason, three different types of lymphatic population are possible (lymphocytes from Peyer's patch, lamina propria and inside the epithelium). The immune enteric system has a more diffi-

cult task than the other mucosa, since it must distinguish not only between self and non-self antigens but also between external antigens, potentially dangerous and normally inoffensive food to whom it is continuously exposed². The enteric immune system is able to execute not only a rapid and effective defence against the outer organisms, but also a specific suppression of the immune response against the non dangerous antigens, to avoid detrimental reactions to the organism (immunology tolerance)³ (fig. 2). The mechanism, assuring the achievement of this balance, is not well known but it interests, at least a part, an accurate selection of specific lymphoid populations and the peculiar cytokines⁴ expression. The immunology tolerance is formed since the beginning of infancy and the role of immune modulation by the enteric flora seems to be essential.

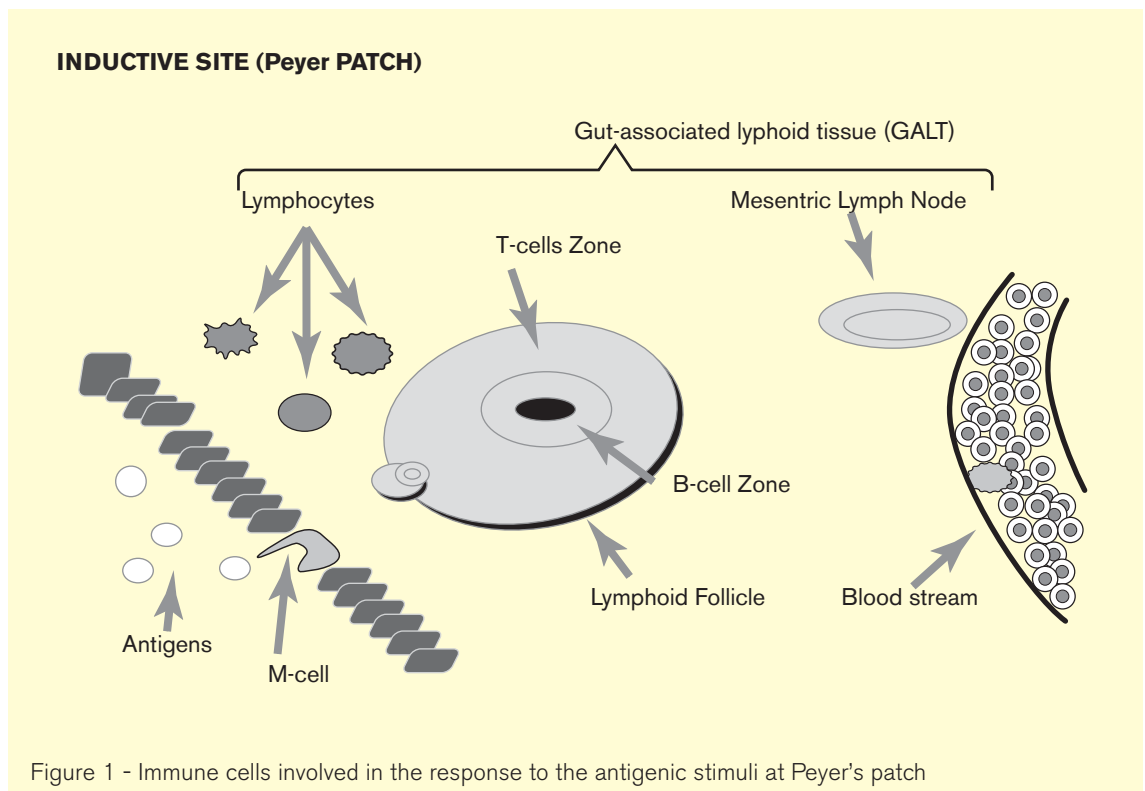


Figure 1 - Immune cells involved in the response to the antigenic stimuli at Peyer's patch

ENTERIC MICROFLORA

The enteric microflora conducts immune response regulation, either local or systemic, promoting a development of lymphoid tissue associated to the intestine within the first months of life⁵. The enteric mucosa, sterile at birth, is successively colonized by different bacteria that constitute a complex ecosystem whose maturation is completed within the first two years of life. Thus a fundamental symbiotic balance between enteric microflora and the host organism is created. Although immune response to enteric microflora is normally considered defensive, this interaction possibly leads to the production of growth factors, cytokines and regulator molecules that control selectively immune function itself. Enteric epithelium's proliferation could be mediated by the T-lymphocytes' activation from food and the enteric microflora⁶. Lymphocytes' activation by bacteria or their prod-

ucts could bring to phenotypic cellular modifications of the enteric epithelium. It's been shown that interferon-g produced by lymphocytes activated to the enteric level, could induce the expressions of immune markers as well as class II molecules from HLA upon the enteric epithelial cells. Indeed, in the newborn, as well as in animals which are germ-free, there is a deficit of expression of HLA II molecules⁷.

Gastro-enteric epithelium is covered by mucous with protective function secreted by goblet cells. Changes in the function of the goblet cells and in the chemical composition of mucous are verified in response to enteric stimuli as well in the alterations of the normal flora. Recent data show that enteric microflora could influence functional dynamics of the goblet cells and mucous compositions: this happens at the local level by bio-activated factors, but also occurs indirectly through the activation of the host's immune system⁸.

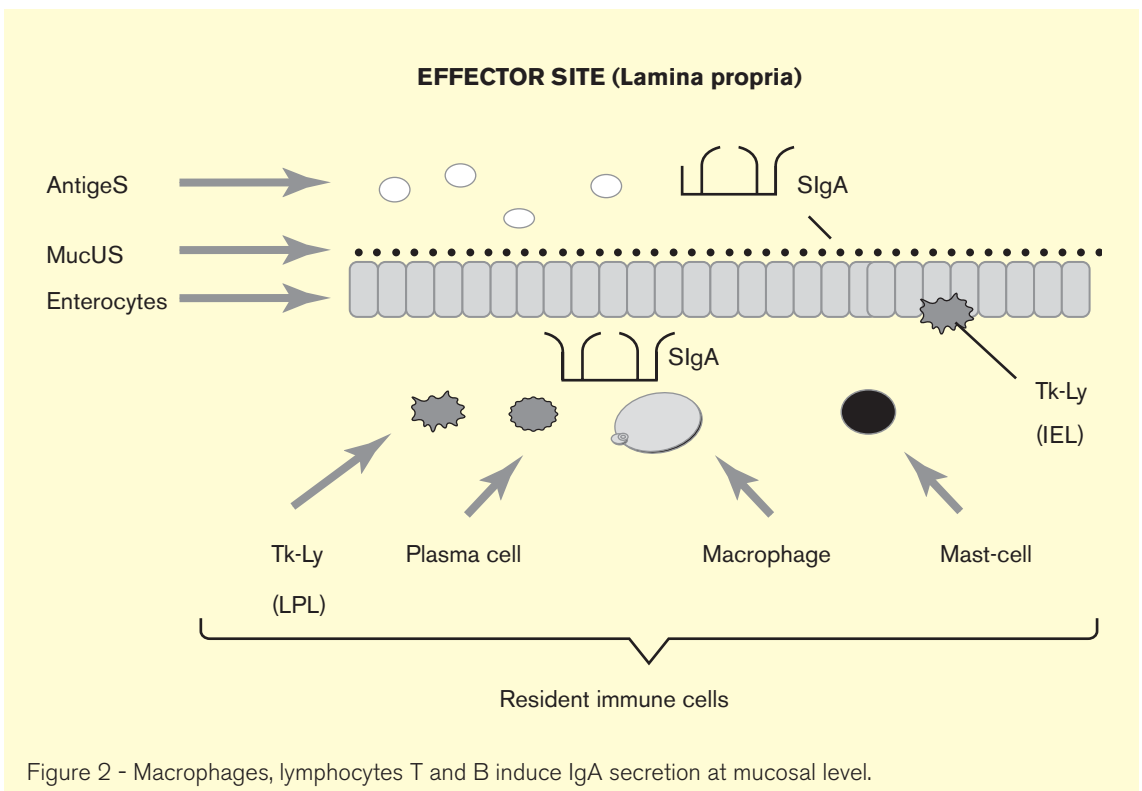


Figure 2 - Macrophages, lymphocytes T and B induce IgA secretion at mucosal level.

HYGIENE HYPOTHESIS

In the context of "Hygiene Hypothesis" there has been proposed that a reduced and different exposition of children to pathogenic and non-pathogenic bacteria in childhood (with consequently alterations of the enteric flora and his turnover) has conduced to immune deregulations and mechanical defensive mechanisms. To confirm this, for example, Sepp et al. have shown that the enteric flora of Swedish children, with a notoriously prevalent rate of allergies, is constituted especially by clostridium, while in the flora of Estonian children, characterised by low allergic rate, lactobacilli and eubacteria⁹ prevail. These organisms have been called probiotics, understood as living microorganisms that, if they are ingested in adequate quantity, exert remarkable benefits to the host¹⁰.

PROBIOTICS AND IMMUNITY

The requisitions necessary for a bacterium stain for being defined probiotic have been codified as followed:

- Human origin
- Absence of side effects in the host organism
- Reproducibility on a large scale in a living form
- Assurance of stability and vitality during *shelf life*
- Resistance to gastric acids and biliary salts
- Adherence to enteric human cells
- Temporary enteric colonization
- Production of anti-microbial substance
- Antagonist against pathogens bacteria

The mechanisms by which the probiotics exert a beneficial action upon the human organism are multiple, but, in certain aspects, they are still obscure. Numerous evidences strengthen the hypotheses that these bacteria favourably influence enteric microbial compositions, the barrier and mucus permeability, as well as the development and the selectivity of lymphoid system as-

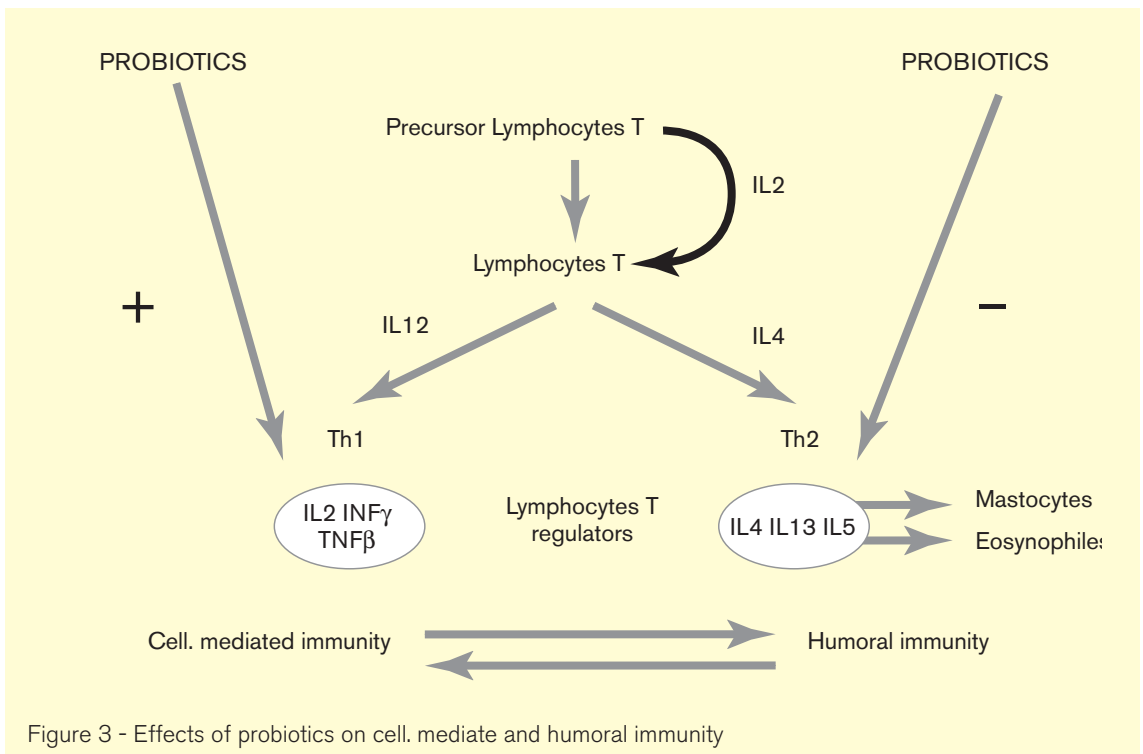


Figure 3 - Effects of probiotics on cell. mediate and humoral immunity

sociated to enteric mucus. It is been, indeed, shown that the probiotics reinforce the different lines of enteric defensive immunity, namely immune-exclusion, immune-elimination and immune-regulation. The first mechanism avails itself of specific factors (secretory IgA and IgM) and non-specific (mucous, peristalsis, proteolysis activity) to reduce antigen uptake¹¹.

Immune-elimination is an inflammatory process that avails itself of inborn immunity (complement, neutrophils, macrophages and mastocytes) to remove noxious agents penetrated in the mucus. Immune-regulation, food and commensal bacteria are tolerated through a phenomenon of hypo-responsorial¹². An efficient enteric flora contributes to this function through exclusion in the competition for nutrients and receptor sites, producing anti-microbial substances and specific antibodies¹³, activating enteric peristalsis through an increase in neuroendocrine cells¹⁴.

Numerous in vitro and in vivo studies confirm that probiotics could have a positive effect not only on the enteric mucous immunity, but also on a systemic level, even if considering cells and cytokines traffic's in lymphatic system and thoracic duct as previously described. Different studies have shown that some lactobacilli stains are able to modulate cytokines pattern of organism in antiatopic sense¹⁵ (Fig.3).

PROBIOTICS, ALLERGIES AND INFECTION

Isolauro's Swedish group have even shown in a randomised double blind study with a placebo group of 27 newborns affected by atopic dermatitis, an increase of SCORAD and a reduction of the cutaneous inflammatory marker (CD4 seric soluble and urinary protein X) after supplementation of hydrolysed milk added with Lactobacilli GG and Bb12 stains. The same

group¹⁶ demonstrated through a two year follow-up of the infants of allergic mothers, that the risk of atopia in children is greatly reduced if milk is enriched with Lactobacillus GG.

Today's most applied probiotics are the Lactobacilli (*L. acidophilus*, *L. casei*, *L. rhamnosus* strani GG, *L. bulgaricus*, *L. reuteri*, *L. plantarum*), biphidocaterium (*B. bifidum*, *B. longum*, *B. breve*, *G. infantis*, *G. animalis*)¹⁷ and some *Streptococcus* stain.

Probiotics are generally distributed on the market as fermented milk and yoghurt. The latter is obtained by keeping milk, whole or skimmed, at 40-45°. Combined with a pure culture of *Lactobacillus bulgaricus* and *Streptococcus thermopiles*, both microorganisms homofermentative are obliged to produce a final product almost exclusively lactic acid from the utilisation of lactose. Regarding fermented milk with probiotics, in the last years we may find different studies in literature upon the effect of *Lactobacillus casei* DN-114 001 upon the immune system. It's been demonstrated that it is particularly resistant to gastric and biliary acids and has the potential to separate at the gastro-enteric level, which is able to modify the microenvironment giving protection against infections¹⁸. Indeed, if supplemented into the diet of children for an adequate time, this lactobacillus is able to colonise the intestine permanently, increasing enzyme digestive functions.

Concerning the anti-microbial effect, it is partly due to its capacity in modulating the enteric immune system, by the stimulation of the hypersensitivity retarded, through antibodies formation and the induction of the activity of the NK cells¹⁹, even in particular physically stress status²⁰.

L. casei DN-114 001 demonstrated to be effective against gastroenteritis. A French study of Pedone et al. ²¹, conducted for 6 months on 287 breast-fed admitted in the hospital with 20

months as average age, showed that adding *L. casei* DN-114 001 to the diet reduced diarrhoea gravity in comparison with to the cow's milk (latte vaccino). The same group, in a more recent study²², showed that the dietary supplementation of yoghurt enriched with the same strain determined a reduction in the incidence of diarrhoea episodes in hospitalised children between 6 and 24 months. Agarwal et al did same determination that put in comparison the benefit of a yoghurt added with *L. casei* to the traditional one in a group of 110 children. Another confirmation on the benefit of probiotics in the micro-environmental enteric regu-

lation is given by Szajewska et al.²⁴; this researcher group is in a recent publication sustaining the effective reduction of the acute diarrhoeic episodes' (especially from Rotavirus) in the breast-feds and in children supplemented with LGG stain.

CONCLUSION

Beyond these interesting clinical scientific evidences, the complex molecular mechanism through which probiotics influence the immune system is largely unknown. Consequently, in fact, further randomised and controlled studies are necessary to confirm and clarify the numerous aspects of this interaction.

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