Review

Probiotics and their Immunomodulatory Potential

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ABSTRACT: The definition of probiotics has evolved from a live active culture which improves the balance of the gut microbiota composition to specific effects, in particular, the immunomodulatory potential of clearly defined strains. The strains with beneficial properties, potential sources of probiotics, most frequently belong to the genera Bifidobacterium and Lactobacillus, and some of these strains exhibit powerful anti-inflammatory properties. These effects are attributed to the normal restoration of increased intestinal permeability and unbalanced gut microbiota, improvement of the intestine's immunological barrier functions and alleviation of the intestinal inflammatory response. The application of probiotics in pediatric practice currently lies in enhancing these barrier functions in the gut and reducing the risk of diseases associated with their dysfunction. The most fully documented probiotic intervention is the treatment and prevention of acute infectious diarrhea.

KEY WORDS: *probiotic, intestinal microflora, immune modulation, inflammation.*

I. Components of the Intestinal Immune System

The defense systems at the level of the intestine may be divided into three lines of defense: the intestinal flora, the mucosa, the intestinal epithelium and the related immune system.

In addition to the main physiological role in digestion and absorption of nutrients, the gastrointestinal tract and its associated immune system act as a barrier between the internal environment and the antigens, such as food and microorganisms from the external environment.

The integrity of the defense of the intestinal mucosa depends on a number of factors, both at mucosal and at the intestinal lumen level.

The nutrition via the gastrointestinal tract is important in maintaining the structure and function of the intestinal mucosa. The lack of nutritional principles can lead to lower edge"' in the brush ", increased permeability and decreased local immunity. Recent discoveries, such as the gut microbiota is an active constituent of the intestinal barrier, resulted in the introduction of therapeutic strategies to combat intestinal infections.[1,2]

The functionality of the intestinal barrier is on of the main mechanisms of defense in maintaining the integrity of the intestinal epithelium and in protecting from the external environmental factors. Once the functionality of the intestinal barrier is impaired, the bacteria and food antigens can reach the sub-mucosa and may induce inflammatory response leading to inflammatory intestinal disease. Recent data have shown that probiotics can initiate intestinal barrier repair after injury. Escherichia coli Nissle 1917 (EcN 1917) not only prevents mucosal barrier damage, but restores also the mucosal integrity in T84 epithelial cells. [3,4]

II. The Role of Probiotics

Probiotics should have the following key features:

- Must be qualitative in terms of food
- Must be present in the form of living cells, preferably in large quantities prior to ingestion
- Must be stable and remain active throughout the life of the product
- Must provide health benefits to the host. [5]

A lot of assumptions were issued on the mode of action of probiotics as well as more speculations but mainly we need to consider the following:

• Probiotics inhibit the proliferation of pathogenic bacteria by producing organic acids and reducing pH;

• Produce H2O2 and prevent the adhesion of pathogenic bacteria to the intestine's wall;

• Produce metabolites able to neutralize bacterial toxins "in situ" or to inhibit their production. Due to the mitigation of the intra-digestive catabolism, there appears a reorientation of gut microbiota in order to reduce the absorption of the toxic substances such as NH3, amines, indoles and the decrease of the bio-transformations of bile salts and fatty acids into the toxic products;

Through their own enzymes the probiotics increase the digestive use of food as well as the detoxification processes. Improving the digestibility of food intake is achieved by: lactose hydrolysis by β-galactosidase, β-glucan degradation by certain probiotic glucanolitic strains, stimulating endogenous enzymatic activity of microorganisms that allow better assimilation of food, stimulating enzymes that are related to the epithelial cells of the tract digestive (lactase, invertase, maltase);

Probiotics stimulate the production of vitamins generally belonging to group B and determine the increase of the activity of lactase, sucrase and maltase;

They multiply in the digestive tract and destroy pathogenic bacteria;

Reduce microbial catabolism, tending towards a better balance between lactobacilli;

Boosts immunity of the host by acting on cells involved in natural immunity and specific immunity. [20]

III. The Effects of the Probiotics on Natural Defense Systems

1. Effects of the Probiotics nn Gut Microbiota

Evolving in the same environment with bacteria, man has come to establish a symbiotic relationship with some of them, thus developing gut microbiota. This, among other benefits, helps prevent autoimmune processes. Oral administration of probiotics was associated with a decrease in the occurrence of autoimmune processes, especially inflammatory intestinal disease and allergies. Lifestyle changes led to changes in the intestinal ecosystem. [5]

There is experimental evidence supporting the influence of ingested probiotic strains on the composition and metabolic activity of gut microbiota in healthy individuals. The survival of probiotics ingested in different segments of the digestive tract varies from one strain to another. Some strains are rapidly inactivated in the stomach, while specific strains of lactic acid bacteria can pass through the entire gastrointestinal tract in large quantities.

As a result of the concentration in the lumen, they may contribute to the transit of the ecology of the micro-flora, at least in the period in which they are consumed. This specific alteration can be observed a few days after the beginning of the consumption of probiotic formula, depending on the ability of the respective strain to modulate the functioning of the gastrointestinal tract. [5]

Significant changes were observed in the number of bacteria in human faeces after eating specific probiotic strains, resulting in overall increased number of species that promote health (*Lactobacillus and Bifidobacterium*) and a low number of potentially harmful strains (some strains *Clostridium and Enterococcus*). However, the studies only reflect the bacteriological situation of the faeces and do not provide an exact situation of the existing conditions in different parts of the digestive tract or the intestinal mucosa. In addition, many species of intestinal bacteria in fecal samples can be grown on plates. [5]

Animal experiments and in vitro studies have shown that probiotic strains have protective action against adhesion, installation, replication and/or pathogenic action of certain germs, through four mechanisms: [5]

- ***Consumption of specific nutrients,*** which in this way are no longer available for pathogens.
- ***Blocking the adhesion to the sites of specific binding***

Different strains of *Lactobacillus* were tested to assess their ability to adhere to Caco-2 cell cultures and to inhibit adhesion of pathogens such as *Escherichia coli or Salmonella typhimurium*, most likely by the simple steric obstruction at the level of enterocitary receptors for pathogens. [6]

- ***Reduction of the intra-luminal pH***

The bifidobacteria, in addition to other sanogenetic actions (the improvement of vitamin and protidic metabolism) have antibacterial action especially on pathogenic species (coli, Staphylococcus aureus, Shigella, Salmonella, etc.) through the production of short chain volatile fatty acids and substances with antibiotic action.

The lactobacilli produce themselves organic acids, hydrogen peroxide and antibacterial peptides (lactocidin, acidophilin, lactacin B, etc.). Most probiotics are producing lactic acid, which lowers the local pH and thus prevents the growth of sensitive bacteria in acid and renders permeable the outer membrane Gram-negative bacteria. Although many studies have been conducted on animals, the effects of lactic acid bacteria on human subjects remain controversial. However, there is evidence of their usefulness. [7,8,9]

By reducing the pH of the intestinal tract, the probiotics may be able to interfere with the

enzymatic activity of intestinal flora. However the mechanism has not been demonstrated in vivo. [10,11]

Goldin and collaborators have shown that probiotic strains reduce the activity of pro-carcinogenic enzymes produced by the intestinal flora. This effect has been shown in detail in animal models and human studies that measured the activity of certain bacterial enzymes (involved in colonic carcinogenesis) in faeces after ingestion of specific probiotic strains. [12,13] However, these studies only reflect the biochemical conditions from the faeces and do not provide an accurate picture of the situation in the entire colon. Since the pH of the colon cannot be measured, it is difficult to evaluate in vivo the effects of acid production by lactic acid bacteria on carcinogenesis. Because of the complexity of developing colon tumors in humans, it is impossible to formulate, on the basis of this result, a benefit in preventing colon cancer. [5]

- ***The production of specific antibacterial substances such as bacteriocines. [14]***

The probiotics compete with noncommensal bacteria and favor their elimination through the secretion of antimicrobial factors, the increased production of antibodies and activation of macrophages and participates in modulating nutrition by producing certain vitamins and fragmentation of undigested molecules. All these features argue in favor of a symbiotic relationship between the human organism and probiotics. This hypothesis would explain in part why administration of probiotics reduces the incidence and severity of autoimmune diseases.

There is epidemiological evidence, which showed that the incidence of autoimmune diseases has increased over the past three decades in industrialized countries, together with a decrease in infectious diseases, which was attributable to antibiotics, vaccines and to the improvement of general hygiene conditions.

2. The Effects of Probiotics on the Intestinal Epithelium

Studies in vitro and in vivo have shown that certain species and bacterial strains produce extracellular glycosidase which degrades the glycoproteins or intestinal mucins and that others are able to stimulate the secretion of mucus, although specific data on probiotics are relatively sporadic. [5,15,16,17] Mack et al. demonstrated by in vitro study, a direct effect of probiotics by the induction of the gene expression in intestinal mucins in epithelial cells, caused either by cell wall determinants or the products of secretion of probiotic bacteria.

The biological role of changes in the expression of complex glycoconjugates and mucus is not yet established. Bernet and collab. have shown that the biochemical changes induced by probiotics could inhibit the binding of pathogenic bacteria in the intestinal cell cultures in vitro. The modifications specific to the mucus layer might be beneficial (by destroying receptors for certain pathogens) or harmful (by exposing receptors covered by mucus layer, providing a place to anchor pathogens in the intestinal wall). [18]

Madsen et al. have shown that some probiotics produce metabolites that directly affect the epithelial permeability and enhance the permeability barrier in vitro. The evidence from studies both on animals and on people shows that the probiotics can restore damaged epithelial permeability and also support the existence of such a mechanism. [19] This ability can protect the host against bacterial translocation and invasion of pathogenic bacteria.

3. The Effects of Probiotics on the Immune System

Intestinal microbial changes following dietary changes may affect the mucosal immune system. The bacteria of the digestive tract form a protective barrier that prevents colonization by pathogenic organisms. This barrier can be affected in various pathological circumstances or ill-judged antibiotic treatments and it is considered that it can be restored by ingestion of live bacteria, even if they temporarily colonize the intestine (which requires their regular consumption). In addition to the barrier effect, several metabolites of lactic bacteria can inhibit the growth of pathogen germs.

Probiotics affect non-specific immunity. It includes two systems: a system that works by antibodies secreted by B lymphocytes (umoral immunity) and another operating system through direct T lymphocytes (cell mediated immunity). The two systems communicate with each other through chemicals called interleukins.

The increase of the specific immune response translates into an activation of T and B lymphocytes which causes an increase in the level of interleukins and circulating antibodies (*immunoglobulin M and immunoglobulin G*).

The probiotics also have an effect on the production of antibodies (mainly *immunoglobulin A*) in the intestinal lumen. In contact with antigens present in the digestive

content, the immunoglobulin A is very important in the digestive tract, representing a first defense against infection. They are produced by the plasma cells of its own lamina, transported through the epithelium and secreted into the intestinal lumen as secretor IgA in combination with a secretor compound.

Immunoglobulin A can inhibit the adhesion of pathogenic bacteria in the mucosal surface of the digestive tract by:

- the agglutination of bacteria
- setting adhesive proteins present on the surface of bacteria;
- in the interference of the adhesive complex substances / cell receptors. [20]

It is known that phagocytosis is accomplished mainly by macrophages and this is the main nonspecific defense mechanism of the body upon penetration of foreign substances. The probiotics stimulate the activity of macrophages. In human studies, it was shown that the production of cytokines, the activity of macrophages, the antibody production and natural killer (NK) cell population can be stimulated by eating yogurt. [21,22,23,24,25]

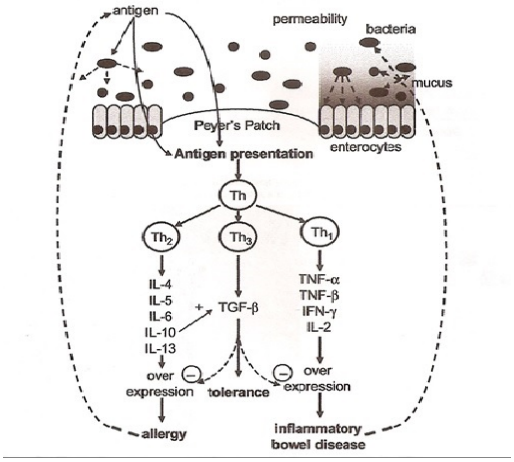

Figure 1. The generation and maintenance of the gut barrier and the anti-inflammatory tone of the immune regulation in the gut by probiotics. Adapted from Reference [30]

The probiotics of *Lactobacillus* strais inhibit the secretion of *the tumor necrosis factor TNFα,* a pro-inflammatory cytokine produced by murine macrophages. Schultz has shown that these may be recognized through TLR2 by antigen-presenting cells in Peyer plates and can stimulate the production of cytokines such as TNFα and IL8. Several Lactobacillus strains can inhibit the proliferation of CD4+ lymphocytes without affecting the production of TNFα, IL4, IL5 and IL10, both in healthy individuals and in patients with inflammatory intestinal disease. [26].

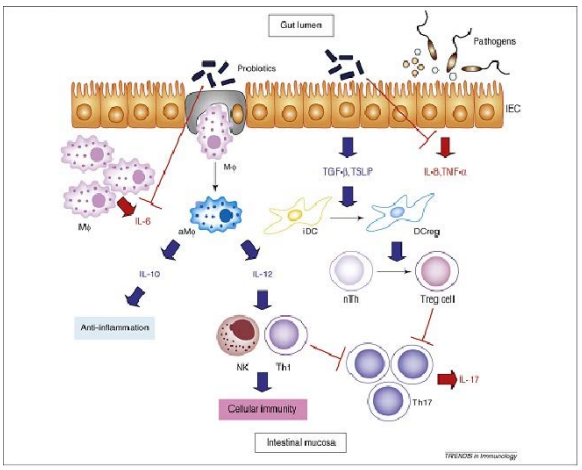

Figure 2. Supposed immune cell circuits in the intestine modulated by probiotics.[31]

When intestinal epithelial cells (IEC) are exposed to pathogen microorganisms they produce pro-inflammatory mediators such as IL8 and TNFα. The probiotics inhibit the production of these cytokines and induce the production of anti-inflammatory mediators such as TGFβ and TSLP (thymic stromal lipoproteins), which can stimulate the differentiation of immature dendritic cells (DCs) in regulatory dendritic cells (DCreg). [27]

The probiotics may determine and direct the differentiation of DCreg, followed by the induction of regulatory T cells (Treg). These ones exercise an anti-inflammatory function by controlling cell Th1, Th2 and probably Th17. The macrophages (M) in the inflamed intestinal mucosa produce large amounts of IL6. [27]

The probiotics may determine the reduction of this production and the increase of the production of IL10. The intestine usually produces small quantities of IL12, but probiotics have the potential to increase this production dependant on the local environment. It is possible that by this mechanism to stimulate the differentiation of CD4+, increase the activity of the cells "natural killer" NK, resulting a local intestinal defense by cytolysis. [27]

The evidence of the immune-stimulating effect of fermented milk products was emphasized by Perdington in 1995. After the ingestion of lactic acid bacteria species by laboratory animals a significant increase in IgA was noticed and the direct yogurt injection into the peritoneum caused the production of a cytokine, type I interferon. Responsible for this effect is bacteria *L. acidophilus*.

Mice who consumed probiotic yogurt were subjected to a translocation of the lactobacillus in the mesenteric nodules, with stimulation of

the ganglionic lymphocyte. Also, the study of Calder in 2002, conducted on mice showed that lactic acid bacteria administered per os increased the number of T lymphocytes, CD4+cells and antibodies, have increased the secretion of IgA, the phagocytosis and respiratory macrophages, as immediate response; they stimulated the lymphocyte proliferation, the NK cell activity, the production of interleukins, interferon and tumor necrosis factors.

Obviously, not all lactic acid bacteria were equally effective, best performance was found in B*ifidobacteria*, *Lactobacillus acidophilus* and *casei* (*Lactobacillus GG*). Research conducted at the Pasteur Institute in Kyoto have shown, for example, that adults who consumed *Lactobacillus brevis* showed a 65% increase interferon levels after 2 weeks of use, and NK lymphocytes activity increased by 68%. And in healthy children, who ate dishes prepared with bifidobacteria, there was also an increase in the total IgA and those of the antipoliovirus. Administration of probiotic bacteria led to lower incidence and severity of diarrhea of different types of institutionalized and hospitalized children [28], associated with the increase of IgG, IgA and IgM anti-rotavirus.

A study made in China by Leyer in 2009, showed that daily supplementation with probiotics is useful in preventing respiratory virus diseases during winter in healthy small children. The probiotics inhibit the adherence of pathogenic enterobacteria (*E. coli*) which considerably reduces the number of cells containing immunoglobulin A. The places of adhesion for lactobacilli and E coli are not the same and the exact mechanism of action is referring either to the direct control of microorganisms, either to the indirect action by stimulating the indigenous micro-flora and their metabolism. Immediately after birth, there can be implemented types belonging to the indigenous normal micro-flora.

It is interesting to relate recent French research conducted on children with *Bifidobacterium longum*, anaerobic bacteria (Gram positive) and that is a normal inhabitant of the intestine of children, adults and animals.

The milk fermented by *Bifidobacterium longum* gave better results than yogurt against diarrhea in children, after the use of the antibiotic erythromycin; more than that, the fermented milk is active against *Rotavirus* infection. The most relevant fact of this experiment is primarily that it has a positive activity in reducing *Escherichia coli and Clostridiu*m *perfringens* by metabolites (glycoprotein) formed during fermentation of milk with *Bifidobacterium longum*. It is important to note that *Bifidobacterium longum* requires the following growth factors: lactose, N-acetylglucosamine or derivatives, peptides and glycopeptides.

Ramond in 1989 reported that *Bifidobacterium* types adhere to the mucosa and β receptors - glucosamine as well as some types of pathogenic *E. coli*. *Bifidobacterium longum* is apparently difficult to implement if the number of indigenous germs is 10^{11} / g.

Specific strains of gut microbiota contribute to the processing of dietary antigens in the intestine to reduce their immunogenicity in vivo and in vitro. For example, the casein degraded by enzymes derived from probiotics has been shown to modulate cytokine production by CD3, in infants with allergy to cow's milk proteins. The recent experiments and studies have shown that specific strains of probiotics inhibit the T cell proliferation and reduce both the secretion of Th1 and Th2 lymphocytes inducing the development of T cell populations producing TGF-β (transforming growth factor) and IL10. [2,29]

However, the exact role of the modulation of the immune response for clinical response to probiotics is still unelucidated. It is difficult to establish certainly, because it is not known exactly how the immune system recognizes and responds to enteric Gram positive bacteria (eg. *Bifidobacterium and Lactobacillus*).

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