Lactic acid bacteria, probiotics and immune system

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ABSTRACT: Mucous membranes of the body are in direct contact with the outside environment and they are colonised by a large number of different bacteria. Through mucous membranes, the organism is in permanent contact with different antigens. Mucous surfaces are protected by many defence mechanisms that ensure a permanent and effective protection. They include the production of secretory IgA, the production of mucus, cytoprotective peptides, defensins etc. Indigenous microflora markedly affects the structure of the host mucous, its function, and the development of the whole immune system. Protective microflora prevents pathogens from adhering by competition for substrates and places of adhesion, and they simultaneously produce antibacterial substances and stimulate the production of specific antibodies and mucus. The early colonisation of the gut with living micro-organisms is important for the development of the gut protection barrier. The number of immune and epithelial cells increases. Probiotic micro-organisms including lactic acid bacteria (LAB) positively influence the composition of the gut microflora; they stimulate the production of secretory IgA; they affect the targeted transportation of the luminal antigens to Peyer's patches and they increase the production of IFN-γ. LAB stimulate the activity of non-specific and specific immune cells. These properties of the LAB depend on the particular species or strain of bacteria. These singularities are probably determined by differences in the cell wall composition. LAB belong to a group of beneficially acting bacteria and they are able to eliminate damage to the gut microenvironment; they stimulate local and systemic immune responses and they maintain the integrity of the gut wall.

Keywords: lactic acid bacteria; gut; mucous layer; immunity

CONTENTS

1. Introduction

Mucous membranes are the unique environment where different bacterial species are able to survive and to express their effects. About 10^{14} bacteria of 200 species, 40–50 genera live on these surfaces. 99% of the whole bacterial population on mucous membranes occur in the distal segment of the small intestine and in the proximal part of the colon (Savage *et al.*, 1998).

Microflora of the gastrointestinal tract plays a crucial role in the anatomical, physiological, and immunological development of the host. It stimulates the immune system to respond rapidly to infection with pathogens and through bacterial antagonism it inhibits the colonisation of the gut by harmful or pathogenic bacteria (Cebra *et al.*, 1999).

It consists of species belonging to the families *Bacteroides*, *Fusobacterium*, *Butyrivibrio*, *Clostridium*, *Bifidobacterium*, *Eubacterium,* and *Lactoba-*

cillus. Enteroccocus and *Escherichia coli* constitute less than 1% of all intestine micro-organisms. Anaerobes dominate upon facultative anaerobes and microaerophiles at the ratio of 1000 : 1 (Mestecky and Russel, 1998). A dominant flora represents 90% of the population, essentially composed of bifidobacteria and lactobacilli. The residual or fluctuating flora (less than 0.01%) of the population is more diversified and contains the potentially pathogenic species (Tournut, 1993).

Bacterial colonisation of the intestine undergoes changes depending on age. It is influenced by local immunity, bacterial fixation factors, and the phenomenon of colonisation resistance (Tournut, 1993). Bacterial strains from the neonatal period are replaced during the life by other bacterial strains characteristic of particular specimens and hosts (Jiang *et al.*, 2001). During the first days after birth qualitative and quantitative changes in the composition of the intestinal microflora are observed. At the time of weaning, lactic acid bacteria and coliforms are replaced by obligatory anaerobes (Berg, 1996).

2. Gut microflora and protection mechanisms of the host

Mucous epithelial surfaces such as the gastrointestinal tract or respiratory tract where the host is confronted with a range of different micro-organisms from the outside environment are suitable places for the start of an infection with pathogens. These surfaces are not unprotected. Different defence mechanisms are involved in the permanent and effective surveillance.

The secretory immune system plays an important role in this case. Secretory IgA (sIgA) is the predominant antibody isotype in the intestinal secretions of mammals. Most IgA is derived from subepithelial plasma cells that produce polymeric IgA with a J chain (pIgA) (McGhee *et al.*, 1989). Epithelial cells express polymeric Ig (pIgR) specific receptors. They are crucial for the selective transportation of immunoglobulins to the gut lumen. IgG and IgM immunoglobulin classes are also present in the gut secretions but they vary in amounts and isotypes according to the animal species. SIgA has a lot of function advantages. The dimeric and tetrameric forms of IgA contain 4 to 8 antigen binding sites and have a multivalent "bonus" effect similar to IgM. IgA is more resistant to the action of proteolytic enzymes that occur in gastrointestinal secretions (Brandtzaeg, 1995). According to Kilian *et al.*, (1998) IgA-antigen complexes do not activate the complement with inflammatory outcomes. Thanks to the content of mannan oligosaccharide side chains, sIgA could inhibit the adherence of bacteria with type I fimbriae to epithelial cells regardless of any specific antibody response (Wold *et al.*, 1990).

The typical response of the secretory immune system is the production of specific secretory IgA antibodies (Underdown and Schiff, 1986) against luminal antigens to prevent other later responses on the epithelial surface. This process is called immune exclusion and offers non-inflammatory protection of the mucous membrane. The integrity of the epithelial layer, the production of mucus, glycolipids, cytoprotective peptides, and antibiotic-like substances are other protection systems of the host (Bengtmark and Jeppsson, 1995).

The protective microflora creates the barrier effect against problematic pathogens and produces regulatory factors such as short-chain fatty acids and bacteriocins. This effect also includes the competition for receptors and metabolic substrates.

More marked but less understood is the role of intestinal microflora in the modulation of immune homeostasis. The intestinal mucous layer is considered to be one of the biggest immune organs of the body, where all the types of immunocompetent cells have been identified (Brandtzaeg *et al.*, 1999). It is possible to define anatomically the induction and effector parts of the immune response inside of the mucosal immune system. The main induction places are Peyer's patches localised along the whole small intestine. Lymphoid and accessory cells of Peyer's patches are covered by the follicular epithelium with M cells that serve as antigen processing cells in the intestinal wall (Neutra *et al.*, 1996).

Lymphocyte migration is important for the transport of immunological information between the different compartments of the intestinal immune system. According to Rothkötter *et al.* (1999) the dendritic cells are the first antigen specific cells draining from the intestines after mesenteric lymph node resection, later preferentially T-cells recirculated through the gut wall. After immigration into the intestinal *lamina propria*, the lymphocytes may enter the space between the epithelial cells where they are present as intra-epithelial lymphocytes.

These intra-epithelial lymphocytes (IEL) may collectively constitute up to 27% of the epithelial cell population and 40% of the peripheral T-cell population. A high proportion of these cells is $CD8^+$ (77% in pigs, 24% in sheep). They differ from blood T-cells. For example, they are $CD90^-$, $CD5^-$ and carry an isoform of CD45 not found on peripheral blood T-cells (Tizard, 2000). The separation of the gut wall from 5-day-old pigs resulted in a 10-fold lower total lymphocyte yield compared with adult pigs where 26.8×10^6 intra-epithelial lymphocytes and 35.2×10^6 total lymphocytes per g of tissue were harvested (Rothkötter *et al.*, 1994).

Intestinal epithelial cells (IEC) are immunocompetent cells (Bland and Warren, 1986) under the permanent influence of luminal modulation factors including the products of bacteria. IEC actively participate in local reactions against pathogens (Jung *et al.*, 1995). The immune functions of enterocytes include interactions with environmental factors, enzymatic processing of food antigens, expression of adhesive molecules, expression of MHC class I and class II molecules, presentation of antigens to lymphocytes, production of cytokines – participation in cytokine net reaction, transportation of secretory immunoglobulins and immune complexes with sIgA (Bland and Warren, 1986; Kaiserlain, 1991). They also contribute to the "education" of thymus independent subpopulations of intra-epithelial lymphocytes (Mayer and Stilien, 1987).

Bacterial dependent activation of intestinal epithelial cells requires a direct contact with IEC and probably the interaction of surface molecules. For the initiation of the local immune response and the activation of specific T-cells, the passage of luminal antigens across the epithelial barrier is necessary. Peyer's patches, or other lymphoid aggregates covered with a specialised epithelium layer with M cells are the main place for antigen passage and T-cell activation (Neutra *et al.*, 1996). The other types of immune cells such as macrophages, dendritic cells and enterocytes are also involved in the handling of antigen at the mucosal level.

T-lymphocytes from the intestinal *lamina propria* are continuously under the antigen influence *in vivo* (Brandtzaeg *et al.*, 1989). They are activated (IL2 receptor expression, CD95) and effectively react to infection. Permanent antigen stimulation is responsible for the proliferation, maturation, and migration of T-cells to distant tissues where they act as effector cells in the immune response (Mestecky, 1987).

T-cells produce lymphokines responsible for the aggregation of other types of immune cells (B cells, inflammatory cells) and for the modification of their microenvironment. One of the most important lymphokines is interferon γ (IFN-γ) produced by activated T-cells. It activates effector cells such as macrophages or epithelial cells (IEC) (Coffman *et al.*, 1988; Mosmann and Coffman, 1989).

Murine IEC express MHC class II and ICAM-1 molecules and they present the antigen to T lymphocytes (Bland and Warren, 1986). This function is modified by the physiological or pathological status of the host (Hughes *et al.*, 1991; Mayer *et al.*, 1991). In cattle, most MHC class II molecules are expressed only on B cells and activated by T-cells. In pigs, resting T-cells express MHC class II molecules at about the same level as macrophages (Tizard, 2000). IEC are able to produce *in vitro* a wide range of pro-inflammatory cytokines such as IL-8, MCP-1, TNF-α and GM-CSF if they are influenced by invasive pathogenic bacteria (Jung *et al.*, 1995). IL-8 and MCP are chemokines that attract and activate neutrophils and monocytes. TNF-α activate immune and inflammatory cells and GM-CSF has a synergistic effect on cell activation (Galli *et al.*, 1991). The infiltration of the tissue with inflammatory effector cells to destroy pathogens is always connected with a certain level of tissue damage. Hence the activation is strictly controlled by external signals such as IFN-γ or TNF-α and by surface molecules like CD54 or CD95, which are able to activate or to depress the activation of IEC (Delneste *et al.*, 1998).

There is a clear difference between Gram-negative non-pathogenic bacteria and lactic-acid bacteria (LAB) in their interaction with IEC. In direct interaction with IEC both types of bacteria induce IFN-γ, but the stimulating effect of LAB is restricted to the cellular surface molecule expression (Delneste *et al.*, 1998). The molecular mechanism responsible for these effects of Gram-negative bacteria and LAB is not understood. The ability of Gram-negative bacteria to enhance the expression of IFN-γ receptors on IEC increases the sensitivity of these cells to activation with IFN-γ. On the other hand, LAB can stimulate IEC for consecutive activation with this lymphokine, which is important in local immune homeostasis.

Permanent antigen stimulation of mucosal surfaces could produce inflammatory lesions of the tissue. The homeostatic mechanisms should be active in the mucous layer to prevent such undesirable

effects. Apoptosis as programmed cell death represents one of the homeostatic mechanisms. Most of the T-lymphocytes in *lamina propria* are cells carrying a surface molecule – Fas that transfers apoptotic signals when it reacts with Fas ligand expressed on activated T-cells. Some T-cells also express Fas and Fas ligands that are potentially reactive with IEC or other T-cells (Boirvirant *et al.*, 1996; De Maria *et al.*, 1996).

3. Relationships between host and micro-organism

Interactions between the micro-organism and the host in the mucous layer are characterised by the active presence of both partners. Evolutionary co-existence constituted micro-organisms and the immune system of the host with similar diversity of selection mechanisms (Pincus *et al.*, 1992).

The successful survival of bacteria during the permanent removal of germs from mucous layers led to the colonisation of these surfaces with micro-organisms. Normal microflora influences the structure of the host mucous, its function and the development of the whole immune system (Kramer and Cebra, 1995). This is shown in experiments on animals bred in germ-free conditions (Uribe *et al.*, 1990). Physiological inflammation in the gut after the colonisation of germ-free animals by non-pathogenic microflora is characterised by strong cell infiltration of the intestinal mucous layer. The exchange of epithelial cells is increased, non-specific and specific immune responses are quickly developed (Lodinova *et al.*, 1991). All compartments of the porcine small intestine contain lymphoid cells at birth, but according to Pabst and Rothkötter (1999) the number of IEL increased in germ-free pigs only slightly compared with conventionally bred piglets. They also observed T-cells in the small intestine *lamina propria*, which expressed neither CD4 nor CD8 molecules. The composition of these types of subsets is still present in germ-free pigs at the age of 1.5 months.

Pathogenic bacteria use different mechanisms to infect the gut. The two most important are adherence to the mucous membrane and the production of toxins. Not all pathogens are eliminated by mucous immune mechanisms because of their high binding affinity to surface glycoproteins, or glycolipids of the epithelial cells (Hoepelman and Tuomanen, 1992). The changes occurring after the binding of bacteria to the cell surface originate by violation of the permeability of tight junctions between epithelial cells. Similarly like lymphocytes, bacteria are equipped with specific surface adhesive molecules that enable the bacteria to adhere to eukaryotic cells (Hook and Switalski, 1992; Svanborg, 1994). Bacterial adhesins are fimbrial or non-fimbrial proteins that recognise saccharide units on the surface of the eukaryotic cells or in an intracellular matrix. Some of the bacterial species (*Shigella, Salmonella, Yersinia*) are caught by epithelial cells and the germs do not have to express specific membrane proteins. Some of the pathogens enter the host tissues through M-cell absorption and many of microbial pathogens use the integrin receptors of the host cells for binding (Perdomo *et al.*, 1994; Jones *et al.*, 1994).

4. Immunomodulation of immune response

The intestinal mucous membrane plays an important role in the exclusion and elimination of potentially harmful antigens and micro-organisms and simultaneously provides selective absorption of nutrients (Brandtzaeg, 1995). Antigen exclusion is connected with such factors as the capacity of the mucous membrane to produce sIgA and mucus (Slomiany *et al.*, 1987). Gut microflora participates in immune exclusion. It prevents other bacteria from adhering by competition for nutrients and places of adhesion, it produces anti-bacterial agents, and it stimulates the production of specific antibodies (Bengtmark and Jeppsson, 1995).

Early exposure of the intestine to live micro-organisms and bacterial colonisation together with dietary antigens is very important for the development of the gut barrier (Helgeland *et al.*, 1996; Sudo *et al.*, 1997). Microflora boosts the barrier development through an increase in the duodenal IgA plasmocyte population (Moreau *et al.*, 1987). It increases the number of enteroendocrine cells in the epithelium of the jejunum and colon, which enhances production of secretory IgA and mucus (Sharma and Schumacher, 1995). The results obtained by Cukrowska *et al.* (2001) showed that at the onset of the intestinal colonisation of germ-free piglets with non-pathogenic *E. coli* O86 the number of IgM, IgG and IgA-secreting lymphocytes increased in spleen, mesenteric lymph nodes, and Peyer's patches in the colonised animals compared with germ-free piglets.

It is known that the microflora of the gut stimulates the proliferation of epithelial cells and increases the whole intestinal surface (Heyman *et al.*, 1986) and the colonisation of the gut with commensal microflora affects the development of the immune system. Arguments about gut microflora as an important part of the exclusion mechanisms of the mucous barrier led to the application of new specific bacterial strains as probiotics.

The application of probiotics can influence the microflora composition by increasing the number of lactobacilli and other anaerobes (Salminen *et al.*, 1998). Dietary supply of probiotic bacteria stimulates IgA immune response (Kaila *et al.*, 1992) and the transport of target antigens through Peyer's patches (Isolauri *et al.*, 1993). Peyer's patches are one of the primary sites in the gut mucous membrane where specific immune responses are performed. Luminal antigens are transported mainly to Peyer's patches and they are confronted with antigen-presenting cells carrying MHC class II (Weiner *et al.*, 1994). The targeted transfer of antigens through Peyer's patches is important in the development of the local secretory immune response. It was proved that probiotics stimulate interferon-γ production (De Simone *et al.*, 1986), which also contributes to antigen presentation by the MHC class II expression pathway and stimulates IgA response. Pessi *et al.* (1998) showed that the probiotics influence mucous permeability by rebuilding damaged macromolecule transportation and they are able to positively influence the lesions caused by an inflammatory response on the membrane. At the same time they enhance the membrane permeability and the immune response to antigen.

Probiotic bacteria influence the processing of mucous layer antigens according to the type of dietary content. *Lactobacillus* GG applied together with unhydrolysed antigen increased the transport of degraded molecules, but if the hydrolysed antigen was applied, the transport was reduced. It is noticeable that the effect of probiotic bacteria in mucous antigen degradation is decreased if the amount of dietary antigen is lower (Pessi *et al.*, 1998).

5. Stimulation of the immune system induced by lactic acid bacteria

The gastrointestinal tract is one of the places most exposed to pathogenic micro-organisms and non-viable materials including antigens and carcinogens. Lymphatic tissue associated with the gut plays a crucial role in the local and systemic immune response (Naukkarinen and Syrjanen, 1986) and mediates the migration and homing of the activated cells from the gut to other sites of the body (Ernst *et al.*, 1988). Foreign micro-organisms and cell fragments can penetrate the gut wall by translocation through the epithelial layer or through Peyer's patches. Indigenous intestinal bacteria including lactobacilli are able to cross the intestinal mucous layer and they can survive in the spleen or in other organs for many days where they stimulate phagocytic activity (Deitch *et al.*, 1990). The proliferative responses of spleen cells to concavalin A and lipopolysaccharide were significantly enhanced in mice given different LAB. These cells also produced significantly higher amounts of interferon-γ in response to stimulation with concavalin A (Gill *et al.*, 2000).

Bloksma *et al.* (1979) showed that a viable strain of *Lactobacillus plantarum* applied intraperitoneally to a mouse stimulated the delayed type of hypersensitivity and non-viable bacteria had an adjuvant effect. In another study, intraperitoneally applied *Lactobacillus casei* activated macrophages (phagocytic capacity and enzyme activity) and natural killer cells (Kato *et al.*, 1984). Subcutaneous inoculation of *Lactobacillus casei* stimulated the production of specific antibodies against *Pseudomonas* antigens by increasing the circulating IgM antibodies (Saito *et al.*, 1983).

Oral application of lactobacilli led to macrophage and lymphocyte stimulation and to the release of the enzymes from murine peritoneal macrophages (Perdigón *et al.*, 1986). Subsequent studies of Perdigón *et al.* (1990, 1991) showed that perorally inoculated *Lc. casei* activated cells in the gut associated lymphatic tissue, which led to the production of significantly higher anti-salmonella secretory IgA titres in the intestinal fluid. The dose and the length of application of lactobacilli were shown to be very important. Only mice treated with *Lc. casei* for 3 consecutive days obtained the therapeutic effect of the lactobacilli application that correlated with sIgA antibody level. These titres also depended on the number of bacteria applied and *Lc. casei* was considered as an oral adjuvant in the prevention of gut infections.

According to our results (Herich, *et al.*, 1999) the 10-day administration of *Lc. casei* to gnotobiotic piglets experimentally infected with *E. coli* in the same dose was more efficient than the 3-day ap-

plication in the stimulating non-specific immune functions.

The mechanisms that lactic acid bacteria use to affect the immune system and produce immunostimulative effects are unknown. Probably, lactic acid bacteria (LAB) alone or their products are absorbed by M-cells and transported to deeper lying lymphatic follicles where they are checked by immunocompetent cells (Brandtzaeg *et al.*, 1987). Eventually, LAB and their products are transported for immune analysis to systemic lymphatic tissues – mesenteric lymph nodes or the spleen. This theory is supported by Classen *et al.* (1995), who found the adherence and ingestion of orally applied lactobacilli by M-cells in mouse Peyer's patches. LAB were found in Peyer's patches after 6–12 hours and in mesenteric lymph nodes 48 hours after ingestion.

The gut epithelium contains lymphocytes able to produce a wide scale of cytokines and influence the local immune environment (Taguchi *et al.*, 1991; Lefrancois, 1994). It is possible that LAB or their products in the gut lumen could directly impact these cells and stimulate their activity. It is possible that LAB enter the body through unspecific pathways mediated by receptor mechanisms. Interactions of LAB and their products with immunocompetent cells such as macrophages and T-cells can lead to the production of cytokines which have a manifold effect on immune and non-immune cells (Nussler and Thomson, 1992).

LAB could induce the production of cytokines through two possible pathways. Cytokine secretion can be released after antigen presentation to T-lymphocytes. Or cytokine production results from the direct interaction between LAB and immunocompetent cells (Taguchi *et al.*, 1991; Lefrancois, 1994). The presence of specific receptors for peptidoglycan – a compound of the LAB cell wall was manifested on lymphocytes and macrophages (Dziarski, 1991). The ability of peptidoglycan to induce the secretion of IL-1, IL-6 and TNF-α by monocytes was proved by Bhakdi *et al.* (1991), Tufano *et al.* (1991), Heumann *et al.* (1994). The induction of IFN-γ production in lymphocytes was shown in the paper of Tufano *et al.* (1991). Cytokines influence the defence system of the host directly or indirectly. Interferons inhibit virus replication, induce expression of MHC class I and class II antigens, stimulate T-helper lymphocytes, activate macrophages, and force immunogenicity of vaccines (Murray, 1988). IL-1 stimulates T- and B-cell proliferation, IL-6 induces B-cell differentiation to plasma cells, and TNF-a has a cytotoxic effect on tumour cells (Gill, 1998).

Factors that influence the immunomodulative activities of LAB:

a) a wide difference in the ability of LAB to influence the immune system (Perdigón and Alvarez, 1992; De Petrino *et al.*, 1995; Paubert-Braquet *et al.*, 1995)
b) the effect of LAB on the immune system depends on the dose (Kishi *et al.*, 1996)
c) live cultures are more efficient in some aspects of immune stimulation compared with dead bacteria (Portier *et al.*, 1993; Vesely *et al.*, 1995)
d) LAB applied in fermented products induce higher responses compared with cells applied in non-fermented products (Perdigón *et al.*, 1986; Saucier *et al.*, 1992)

Other factors that could influence the efficiency of LAB by stimulation of immunity are probably the age of the host, the physiological status of the host, and the quality and quantity of feeding.

For example, Tejada-Simon *et al.* (1999) did not record any positive effects of repeated (up to 14 days) oral exposure to viable and nonviable lactic acid bacteria on the basic cytokine mRNA expression in mucosal and systemic lymphoid tissue and on immunoglobulin levels. According to Gill *et al.* (2000) the feeding of *Lc. rhamnosus, Lc. acidophilus* or *Bifidobacterium lactis* in mice had no significant effect on IL-4 production by spleen cells or on the percentages of $CD4^+$, $CD8^+$ and $CD40^+$ cells in the blood although immunostimulative effects were observed in other parameters.

6. Effect of lactic acid bacteria on non-specific immune response

Non-specific immune response constitutes the first line of defence for the host. It is induced by different stimuli and it is quickly activated. The cell base of non-specific immunity is composed of mononuclear phagocytic cells (monocytes, macrophages), polymorphonuclear leukocytes (mainly neutrophils) and NK cells. Phagocytosis initiates a series of intracellular reactions that continue with the production of reactive oxygen and nitrogen radicals, TNF-α and IL-1 (Tizard, 2000).

The application of some strains of LAB produced:

a) enhanced activity of peritoneal and pulmonary macrophages and blood leukocytes (Moineau

and Goulet, 1991; Paubert-Braquet *et al.*, 1995; de Petrino *et al.*, 1995)

b) increased secretion of lysosomal enzymes (Paubert-Braquet *et al.*, 1995; Perdigón *et al.*, 1988), increased production of reactive oxygen, nitrogen radicals, and monokines of phagocytic cells (Balasubramanya *et al.*, 1995)

c) *in vivo* enhanced clearance of colloidal carbon as an indicator of the phagocytic ability of the monocyte-macrophage system (Perdigón *et al.*, 1988).

The results from different studies on animals show that the increased function of phagocytic cells depends on the species or strain of bacteria. The secretion of lysosomal enzymes in macrophages by mice fed fermented milk with *Lactobacillus casei* was more effective compared with the feeding of fermented milk containing *Lc. acidophilus* and *Streptococcus termophilus* (Perdigón and Alvarez, 1992). Tortuero *et al.* (1995) found an increase in the interleukin-2 concentration in the ileal tissues in piglets treated with *Streptococcus faecium* M-74 and *Lc. casei* spp. The histological structure of the epithelium of this group of piglets showed an increase in the phagocytic activity of the cells. The differences in the cell-wall composition are probably responsible for the different action of several lactobacilli strains. Moreover, the strains able to survive in the gastrointestinal tract, able to adhere to the intestine mucous membrane, and able to persist at the critical limit are more effective in stimulating phagocytic cells (Perdigón and Alvarez, 1992; Schiffrin *et al.*, 1997). It is remarkable that fermented products expressed better stimulation of the non-specific immune system probably due to immune-active peptides formed during fermentation from milk proteins (Fiat *et al.*, 1993).

7. Effect of LAB on specific immune response

The specific immune response could be divided into two main categories: humoral immunity and cellular immunity. Antibodies produced in plasma cells (mature B-lymphocytes) mediate humoral immunity. Cellular immunity is mediated byT-lymphocytes, which proliferate after contact with antigens, produce cytokines, and influence the activity of other immunocompetent cells (Tizard, 2000).

Many authors assume the protective effect of lactic acid bacteria against gut infections and tumours (Adachi, 1992; Aso *et al.*, 1995; Saavedra, 1995). The induction of a mucosal immune response is not easy due to the development of oral tolerance, but Perdigón *et al.* (1999) demonstrated that certain lactic acid bacteria are able to induce specific secretory immunity, and others will enhance the gut inflammatory immune response. *Lc. casei* and *Lc. plantarum* were able to interact with Peyer's patch cells and showed an increase in IgA^-, $CD4^+$ cells, and antibodies specific for the stimulating strain. *Lc. lactis* and *Lc. delbrueckii spp. bulgaricus* induced an increase in IgA^+ cells entering the IgA cycle but not $CD4^+$ cells. Nader de Macias *et al.* (1992) described the increased resistance to *Shigella* infection mediated by high titres of anti-*Shigella* antibodies in serum and in intestine secretions in mouse fed fermented milk.

In the experiment of Herías *et al.* (1999), gnotobiotic rats given *Lc. plantarum* in addition to *E. coli* showed lower counts of *E. coli* in the small intestine and caecum one week after colonisation compared with a group colonised with *E. coli* alone. Rats colonised with *Lc. plantarum* had significantly higher total serum IgA levels and marginally higher IgM and IgA antibody levels against *E. coli* than those colonised with *E. coli* alone.

Perdigon *et al.* (1990) found a correlation between the increased immune response and resistance to *Salmonella* infection in mice fed milk cultures. Similar observations were recorded by Paubert-Braquet *et al.*, (1995) in the case of the host resistance to *Salmonella typhimurium*.

The application of *Lc. casei* and *Lc. bulgaricus* terminated corticoid-induced immunosuppression in mice with *Candida albicans* infection (De Petrino *et al.*, 1995). The animals inoculated with lactobacilli showed a significant increase in specific and non-specific immune responses and they reached higher levels of antibodies compared with non-immunosuppressed control animals.

According to Alvarez *et al.* (1998) viable culture of *Lc. casei* protects the host against *Salmonella typhimurium* infection not only after the first application, but it also maintains the effect after a simple revaccination on day 15 or 30 after the first application. They estimated that the protective effect was obtained when the number of IgA secreting cells in the *lamina propria* of the intestine and the level of secretory IgA in the gut fluid increased. In correlation with this fact, they observed an increased number of polymorph nuclear cells that induce an inflammatory immune response and influence the mucous membrane integrity.

From this point of view, it is important to maintain the levels of $CD4^+$ and $CD8^+$ T-lymphocytes. The population as a whole could be increased, but the balance between them should be retained. An increase in only the $CD8^+$ T-cell population of T-lymphocytes in the *lamina propria* could induce an inflammatory response probably through the cytotoxicity of these cells. On the other hand, a one-sided increase in the number of $CD4^+$ T-lymphocytes, particularly Th1 population, could induce an enhanced expression of HLA class II through the cytokine pathway (Lionetti *et al.*, 1995) followed by a higher capture of antigens and the overstimulation of the mucous. According to Brandtzaeg (1996) the immune overstimulation in the gut is already produced through $CD4^+$ T-cell pathways.

According to Havenith *et al.* (2002) lactobacilli are known as safe bacteria and they have a number of properties that render them highly suited as vehicles for the delivery to the mucosa of compounds that are of pharmaceutical interest. The immunomodulating capacity of lactobacilli together with the possibility of targeting antigens to specific sites of the bacterium offers attractive opportunities for the treatment of infectious diseases, auto-immune diseases, or other immune disorders by modulating the immune response in a directed and predetermined way.

8. Conclusions

Different mechanisms could influence the composition of the micro-organisms that colonise the digestive tract. The two important are: antagonism among bacteria and local immunity. Disturbances in the ecological balance in the gut lead to the growth of harmful bacteria and to their possible translocation to internal organs, which induces disease.

Beneficially acting bacteria positively influence the immune system of the host. The protection of the mucous membranes is ensured through local immunity defence mechanisms. Their development is dependent on the direct contact of the host with antigens from the outside environment. The indigenous microflora joins in immune exclusion and protects the host from the adhesion of pathogens through competition for substrates and places of adhesion. These bacteria produce antibacterial substances and they stimulate the production of specific antibodies. LAB is one of the groups of bacteria that occur physiologically in the digestive tract of mammals. These bacteria influence the distribution and the numbers of lymphoid cells in lymphatic tissues associated with the gut, ensure the balance in the composition of the gut microflora, and through their activity are able to maintain the integrity of the gut mucous membrane.

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