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RETHINKING THE ROLE OF PROBIOTICS FOR THE PREVENTION AND TREATMENT OF ENTEROPATHIES

CLAUDIO DE SIMONE[1], GIUSEPPE FAMULARO[2], EMILIO JIRILLO[3], RENATA VESELY[4], BRUNA BIANCHI SALVADORI[4], and GINO SANTINI[1]

[1]Infectious Diseases, and [2]Internal Medicine, Department of Experimental Medicine, University of L'Aquila; [3]Department of Immunology, University of Bari; and [4]CSL, Milano; Italy.

SUMMARY

In this paper we review the experimental and clinical data indicating that lactic acid producing bacteria of the normal endogenous intestinal microflora strongly affect most functions of immune system, particularly at the level of gut-associated lymphoid tissue (GALT), including the production of cytokines, the mitogen- and antigen-driven proliferation of lymphocytes, the cytotoxicity of natural killer cells, and the production of antibodies. Furthermore, via these mechanisms as well as via exerting a barrier effect, lactic acid producing bacteria counteract the translocation of Gram-negative bacteria from the gut. Furthermore, lactic acid producing bacteria have been shown to mediate the pathogenesis of autoimmunity in experimental models, such as the murine model of Kawasaki disease.

Recent reports have also demonstrated that the administration of lactic acid producing bacteria could prove helpful in the treatment under *in vivo* conditions of enteric infections from pathogens, such as *Salmonella* spp. and rotaviruses. Finally, lactic acid producing bacteria have been shown to reduce mutagenicity in some experimental models, therefore suggesting a role for probiotics in the prevention of malignancy.

INTRODUCTION

The endogenous bacterial microflora have a key role with regard to many metabolic functions and in natural resistance to infections from several pathogens, mostly in the gastrointestinal tract.

A body of evidenc indicates that lactic acid producing bacteria, which mainly account for the endogenous intestinal microflora, may strongly affect most functions of the immune system, particularly at the level of the gut-associated lymphoid tissue (GALT), including the production of cytokines, the proliferation of lymphocytes following mitogens and antigens, the cytotoxicity of natural killer (NK) cells, the production of antibodies, and the metabolic and phagocytic functions of macrophages.

Here we will briefly review the physiology of endogenous intestinal microflora and the most common and significant conditions leading to distur-

bances in the normal intestinal microecology. Furthermore, since a strict relationship linking foods containing lactic acid producing bacteria, such as yoghurt, the endogenous bacterial microflora, and the regulation of immune response toward pathogens has been suggested, we will summarise both clinical and experimental reports emphasising the use of lactic acid producing bacteria in therapy and immunomodulation of human diseases. Overall, the reviewed data indicate that lactic acid producing bacteria could be a promising tool for ecological therapy of mucosal diseases as well as for the development of original and flexible vectors for targeting in the gastrointestinal tract.

CAUSES OF MICROFLORA DISTURBANCES

The most common and significant cause of disturbances in the normal endogenous intestinal microflora is the administration of antimicrobial agents (*Lidbeck* and *Nord*, 1993; *Nord*, 1993). The administration of these agents may seriously disturb the balance of the normal intestinal microflora. This disturbance can cause bacterial overgrowth and the emergence of resistant microorganisms which may lead to serious infections and also encourage the transfer of resistance factors among bacteria.

Many antimicrobial agents, including penicillins, cephalosporins, monobactams, carbapenems, macrolides, clindamicin, tetracyclines, nitroimidazoles and quinolones, may have ecological effects on the human intestinal microflora (*Lidbeck* and *Nord*, 1993; *Nord*, 1993).

Laboratory animals are a valuable model which allows us to evaluate the effects of antimicrobial administration upon intestinal bacterial microflora. For example, in mice the selective elimination of aerobic Gram-negative bacteria by oral polymixin, aztreonam or temocillin resulted in a reduction of the endotoxin concentration of faecal supernatants to 10% of untreated controls (*Goris* et al., 1986). In addition, further decrease of the endotoxin concentration to 1% was achieved by total decontamination of the intestinal tract by oral cephalotin/neomycin treatment (*Goris* et al., 1986).

In an experimental rat model (*Minelli* and *Benini*, 1993), fluoroquinolones proved able to reduce the levels of enterobacteria, while Gram-positive bacteria (enterococci, staphylococci, lactobacilli) were little affected. It is worth noting that comparable effects were observed after intraperitoneal and oral administration of oral pefloxacin. The changes induced by fluoroquinolones on intestinal flora showed a uniform trend: certain differences may be ascribed to different pharmacokinetic properties such as bioavailability and metabolism. In this model, parenteral imipenem caused a significant decrease in the mean concentrations of *E. coli*, clostridia, and fungi, whereas aztreonam induced a marked and prompt inhibition of *E. coli* and *Proteus* spp. The prolonged treatment, in turn, induced an overgrowth of fungi and bacteroides. Teicoplanin caused a significant decrease in the clostridia and anaerobic lactobacilli. These results are closely comparable to those observed in humans (*Minelli* et al., 1993).

The potential of an antimicrobial agent to change the intestinal microflora is related to its antibacterial activity, route of administration and pharmacokinetic properties, such as incomplete absorption of any orally administered antibacterial compound, secretion in the

bile or from the intestinal mucosa (*Lidbeck* and *Nord*, 1993; *Nord*, 1993). Notably, in most cases the parenteral route induced changes in the intestinal ecosystem as did oral administration.

In subjects with gastrointestinal inflammation (gastritis, duodenitis, enteritis, cholangitis, cholecystitis) the microbial flora may be qualitatively and/or quantitatively modified with no clinical and functional repercussions (*De Simone* et al., 1993). However, after episodes of acute diarrhoea frequently one could find a reduction of anaerobic agents concurrent with the causative agent (*De Simone* et al., 1993).

Also in patients with inflammatory bowel disease, most notably ulcerative colitis, the faecal microflora undergoes changes (*Yamamura*, 1987). In fact, the counts of obligate anaerobes were significantly decreased irrespective of stool condition, stage, and severity of the disease. The frequencies of detection of obligate anaerobes were much lower particularly in severe cases and cases with bloody diarrhoea. Specifically, the counts and frequencies of detection of bifidobacteria reflected the severity of the disease (*Yamamura*, 1987). Furthermore, in the genera of the Enterobacteriaceae *E. coli* was not isolated and *Proteus*, which was not isolated from the faeces of healthy individuals, was often isolated and became dominant in severe cases (*Yamamura*, 1987). These findings clearly point out the presence of abnormal microflora in the gut of patients with ulcerative colitis. In addition, facultative anaerobes, such as streptococci, were increased more markedly in first relapsing cases as compared with untreated cases (*Yamamura*, 1987).

High doses of radiotherapy may cause an abnormal proliferation of bacteria, chiefly focal, which is related to the absorbed amount of radiation (*De Simone* et al., 1993).

In liver cirrhosis, it has been demonstrated that an abnormal bacterial increase in the stool is associated with poor absorption (*De Simone* et al., 1993). In these patients, the microflora of the intestine acquires pathogenic properties because of the products of its metabolic degradation. In fact, due to the reduced liver capability of detoxification the amino acid metabolites (ammonia, indoles, phenols, amines) pass through systemic circulation without being detoxified properly. It remains to be established whether the bacteria in the intestine of patients with liver cirrhosis are able to produce larger amounts of ammonia than in other conditions of increased bacterial proliferation or, in turn, this phenomenon could be related to a percent increase in *Klebsiella* and *Proteus* counts which contribute to a great extent to the derangement in urea metabolism.

Finally, since enteric infections have been implicated in the heterogeneous pathogenesis of the irritable bowel syndrome (IBS), which includes factors ranging from psychoneurotic behaviour and emotional stress to dietary fibre deficiency and food intolerance, and there is evidence of an increase in the inflammatory cells present in the gut of some IBS patients (*Collins*, 1992; *Whitehead* et al., 1992), it could be hypothesised that the immune reactivity toward modification in the endogenous intestinal microflora could have a role in the pathogenesis and pathophysiology of at least a subpopulation of IBS patients.

COLONISATION RESISTANCE AND LACTIC ACID PRODUCING BACTERIA.

Low *Bacteroides* counts in the stool, for instance, have been correlated with an increased susceptibility to infection from *Salmonella enteritidis* as well as combined *Escherichia coli*, whereas *Proteus mirabilis* and *Streptococcus faecalis* have been shown to antagonise *Vibrio cholerae* (*De Simone* et al., 1993). The protection afforded by microflora against intestinal colonisation with pathogens is referred to as colonisation resistance. It is worth noting that this mechanism of natural resistance is involved also in protecting the host against fungal infections, in addition to bacteria, as shown by the resistance afforded by *E. coli* toward *Candida albicans*, regardless the immunocompetence of the host (*De Simone* et al., 1993).

The healthy human body harbours an extensive number of microrganisms that inhabit surfaces and cavities exposed or connected to the external environment. It is estimated that the intestinal microflora of any given individual contains more than 400 species of bacteria. The major bacterial populations are located in the large intestine where the bacterial concentration is 10^{11} to 10^{12} CFU/ml of faecal material (*Simon* and *Gorbach*, 1986). Notably, the microflora is not a static population but its composition is the result of host physiology, microbial interactions, and environmental influences, including the steroid sex hormone pattern and diet (*Finegold* et al., 1974; *Marsh* et al., 1992; *Minelli* et al., 1993).

Although the host plays a direct role in colonisation resistance by desquamation of mucosal cells, secretion of saliva or mucus, and finally by swallowing or peristalsis, a relevant contribution of the host may come more indirectly both through the gut-associated lymphoid tissue (GALT) and endogenous bacterial microflora. Recently, it has been tentatively concluded that the GALT may not only respond positively to foreign (bacterial) antigens by producing specific IgA but also by forming specific suppressor cells. The induction of specific unresponsiveness (tolerance) to certain microrganisms may explain the stability of the composition of the endogenous flora. It may also explain the difference in intestinal endogenous flora between individuals of the same species. This hypothesis is supported, at least partly, by the finding that during circumstances of significantly reduced thymus function since birth, important components of the endogenous flora and thus of colonisation resistance are apparently inhibited (*van der Waaij*, 1985). This, and the malfunction of the T cell-depleted GALT, allows potentially pathogenic microbes to colonise the gut in abnormally high numbers and great diversity, a condition which is associated with a clinical syndrome called "wasting disease".

Furthermore, recently considerable evidence has been accumulated showing that various members of physiological microflora liberate low molecular weight peptides which, apparently, are essential for adequate immune responses of the host (*Pulverer* et al., 1993). The antibiotic decontamination (e.g. of the BALB/c mouse intestinal tract) results in a lack of generation of immunopriming microbial peptides leading to immunosuppression (*Pulverer* et al., 1993). The biochemical analysis revealed reproducible chromatographic fractions which selectively influence maturation, proliferation, and activation of immune cells (*Pulverer* et al., 1993).

BACTERIAL TRANSLOCATION AND LACTIC ACID PRODUCING BACTERIA.

Bacteria constituting the endogenous intestinal microflora may pass still alive through the gastrointestinal lumen and reach the local lymphatic organs (Peyer's patches, mesenteric lymph nodes) as well as other organs (liver, spleen, blood). This phenomenon occurs more frequently with Gram-negative bacteria and facultative anaerobes (*E. coli*, *P. mirabilis*, *K. pneumoniae*) than with obligate anaerobes and Gram-positive bacteria. Such a phenomenon does not occur under physiological conditions when an intact endogenous intestinal microflora is present and the host is fully immunocompetent (*De Simone* et al., 1993). Usually, viable endogenous bacteria are not detectable in the organs of healthy gnotobiotic animals since the translocating microrganisms are killed while in transit or upon arrival in the reticuloendothelial organs (*Berg*, 1988). Since endogenous bacteria translocate from the gastrointestinal tract at higher rates in neonatally thymectomised mice or nu/nu mice than in nu/+ or thymus-grafted nu/nu mice it was postulated that a high caecal population level of a given strain is not the unique prerequisite for translocation (*Steffen* et al., 1988). In addition, it was shown that immunosuppressive and antimicrobial agents, thymectomy, endotoxin, tumours, diabetes, protein malnutrition, and thermal injury can promote translocation (*Walken* and *Owen*, 1990). Furthermore, in experimental animal models bacterial translocation was found following small bowel transplantation and in animals with graft-versus-host disease (GvHD), with *Staph. epidermidis* being the most prevalent organism (Price et al., 1993). There was a large increase of *Staph. epidermidis* mostly in animals with transplant rejection. These findings demonstrate that GvHD and transplant rejection are associated with shifts in intestinal microflora toward potentially pathogenic organisms and that bacterial translocation leads to a major threat for the development of sepsis.

As a rule, bacterial translocation is due to an altered permeability of the intestinal epithelium after stress, unapparent infections, tumours or to the host's immunosuppression (*Walken* and *Owen*, 1990). Therefore, the bacterial translocation may be considered as either a deficiency of the mucosal barrier in confining the bacteria to the gastrointestinal lumen or a deficiency of the immune system and non-specific defences in clearing and killing bacteria which cross the intestinal barrier or both. This phenomenon could have a key role in the pathogenesis of opportunistic infections due to endogenous intestinal bacteria, mostly in debilitated patients and/or those with tumours, during long-term treatment with either immunosuppressive drugs or antibacterial compounds or both.

It is important to know whether certain foods or bacterial species are involved in modulating bacterial translocation from the gut. For example, commercially available chemically defined liquid diets result in altered intestinal microflora and bacterial translocation from the gut (*Alverdy* et al., 1990) whereas enteral feeding with peptide nutrients has been shown to improve the mucosal barrier against microrganisms (*Alexander*, 1990).

Recent experiments from our groups have shown that *Lactobacillus bulgaricus*, one of the lactic acid producing bacteria present in yoghurt, which has the capability to adhere to the intestinal mucosa (*Bianchi Salvadori* et al., 1984; *Bianchi Salvadori*, 1986), is pivotal in

inhibiting translocation of Gram-negative bacteria present in the gut (*De Simone* et al., 1992). According to our results, the barrier effect exerted by *L. bulgaricus* against *E. coli* translocation approximated >70%. However, our data rule out a simple barrier effect played by *L. bulgaricus* and suggest that *L. bulgaricus* boosts the host's immune defences against translocated *E. coli* (*De Simone* et al., 1992).

MODULATION OF CYTOKINE PRODUCTION BY LACTIC ACID PRODUCING BACTERIA

Endogenous bacterial microflora, mostly lactic acid producing bacteria, have been shown to strongly modulate the cytokine network which regulates the immune response and drives effector arms toward invading pathogens. For example, peritoneal macrophages from normal mice produced significantly more IL-1 and IL-6 *in vitro* than those of germfree mice (*Nicaise* et al., 1993). Furthermore, IL-1 and IL-6 production from germfree mice implanted with *E. coli* was as comparable as in normal mice (*Nicaise* et al., 1993). In turn, *Bifidobacterium bifidum* did not increase the production of these two cytokines (*Nicaise* et al., 1993). In these experiments, TNF-α was produced only by peritoneal macrophages from normal mice and germfree mice implanted with *E. coli* (*Nicaise* et al., 1993).

These data, overall, suggest that Gram-negative bacteria are the most efficient stimulus for driving the production of macrophage derived cytokines. Also, it is worth to note that bacterial flora stimulated cytokine production soon after implantation. In addition, recent reports have demonstrated that *L. acidophilus* induces the production of IFN-α/β by murine peritoneal macrophages (*Kitazawa* et al., 1993), therefore suggesting that the inducing activity of IFNs may be one of the available biologic parameters for designating the diary products containing *L. acidophilus* as physiologically functional foods. Both of the bacteria commonly found in yoghurt (*L. bulgaricus* and *Streptococcus thermophilus*) have been shown to induce the production of IL-1 β, TNF-α, and IFN-γ, but not of IFN-α and IL-2, by peripheral blood mononuclear cells (PBMCs) from humans (*Pereyra* et al., 1993). Furthermore, the walls from these bacteria, but not their cytoplasm, induced a comparable cytokine production (*Pereyra* et al., 1993). These cytokines were also induced by *L. casei*, *L. acidophilus*, *Bifidobacterium* spp., and, to a lesser extent, *L. helveticus* (*Pereyra* et al., 1993). Notably, in this report the IFN production was estimated by the 2-5 synthetase activity from PBMCs following a single ingestion of bacteria in yoghurt or sterile milk and the activity of the yoghurt group enzyme was about 80% higher than that of the milk group; however, no cytokine was detectable in the serum (*Pereyra* et al., 1993).

In a recent report from our laboratory, we have shown that the addition of small quantities of yoghurt containing live *L. bulgaricus* and *S. thermophilus* to ConA-driven human PBMCs resulted in a strong enhancement of IFN-γ production (*De Simone* et al., 1993). Furthermore, these supernatants augmented NK cell cytotoxicity against K562 targets much more with respect to control supernatants from PBMC cultures stimulated with ConA only (*De Simone* et al., 1993). Similar results were found by using *L. aci-*

dophilus, *L. casei*, and *L. plantarum* (*De Simone* et al., 1986). Our hypothesis was that lactic acid producing bacteria induce PBMCs to produce cytokines, such as IL-1 and IL2, which activate resting NK cells to synthesise and release IFN-γ, proliferate, and exert cytotoxicity. This hypothesis has been further supported by experiments demonstrating the binding of lactic acid producing bacteria to both CD4 and CD8 cells (*De Simone* et al., 1993; *De Simone* et al., 1988a). As seen in the case of *Salmonella*-stimulated lymphocytes (*De Simone* et al., 1986; *Antonaci* and *Jirillo*, 1985; *De Simone* et al., 1988a), the binding of lactic acid producing bacteria to T lymphocytes should be referred to as a potent stimulus for immune cell activation.

Recently, our group has shown that also the administration of lactic acid producing bacteria under *in vivo* conditions may strongly enhance the production of IFN-γ. In our study, healthy volunteers have received lyophilised dietary lactobacilli (3×10^{12} microorganisms) and 200 g of plain yoghurt at 24 hours intervals for 28 days. The control group received skimmed milk in a quantity calorically equivalent to that of the yoghurt group. Our results were that by feeding large quantities of dietary lactic acid producing bacteria, a strong increase in the serum levels of IFN-γ as well as the expansion of both B lymphocytes and NK cells can be attained in the normal host (*De Simone* et al., 1993).

However, we have data obtained in an experimental model of *Cryptosporidium parvum* infection indicating that also mechanisms other than the increased production of IFN-γ are involved in mediating the increased resistance toward pathogens induced by lactic acid producing bacteria.

C. parvum is a protozoan parasite that causes diarrhoeal disease in a variety of mammals, including humans and economically important livestock species (*Fayer* et al., 1990). The disease is especially severe in immunocompromised hosts and has become a major cause of morbidity and mortality among patients with the acquired immunodeficiency syndrome (AIDS) (*Ungar*, 1990). Mechanisms of immunity to *C. parvum* are not well understood (*Zu* et al., 1992), but several *in vivo* studies suggest that both CD4 lymphocytes and IFN-γ are critical in resistance and recovery from C. parvum infection (*Ungar* et al., 1991; *Chen* et al., 1993; *Chen* et al., 1993), in addition to endogenous bacterial intestinal microflora. In fact, it is worth to note that germfree adult mice are more susceptible to the primary challenge than normal mice and while severe combined immunodeficient (SCID) mice are relatively resistant to *C. parvum* infection, germfree SCID mice are highly susceptible (*Harp* et al., 1992). Therefore, the presence of intestinal microflora strongly influences mice susceptibility to *C. parvum* infection.

The results of our experiments support the hypothesis that the colonisation of the gut of germfree mice with lactic acid producing bacteria can protect them from *C. parvum* infection (*Harp* et al., submitted). In fact, we found that germfree mice colonised with lactic acid producing bacteria were clearly less infected with *C. parvum* than controls without lactic acid producing bacteria (*Harp* et al., submitted). However, this protection was not directly correlated with induction of IFN-γ by lactic acid producing bacteria (*Harp* et al., submitted) since the two groups challenged with *C. parvum* both produced mRNA for IFN-γ despite the fact that mice colonised with lactic acid producing bacteria were protected from infection and the non-colonised group was not (*Harp* et al., submitted). In addition, in

our experiments germfree mice treated with lactic acid producing bacteria only did not produce message for IFN-γ (*Harp* et al., submitted). Therefore, the colonisation of germfree mice with lactic acid producing must be protecting either via some mechanisms not involving IFN-γ or through an indirect pathway. Additionally, it is possible that IFN-γ message and/or protein was induced earlier in animals receiving lactic acid producing bacteria only and disappeared by time of necropsy while the message persisted in mice injected with both lactic acid producing bacteria and *C. parvum*. It is further possible that the group injected with *C. parvum* only was not protected from challenge because the induction of IFN-γ seen in this group was a late event occurring as a result of infection and was not sufficient to prevent colonisation with the parasite.

The implications of our findings in SCID mice with experimental *C. parvum* infection in other species, such as humans with AIDS, are not still clear. It is interesting that the apparent age-related susceptibility and development of resistance in calves and immunocompetent mice correlates with the acquisition of intestinal microflora (*Harp* et al., 1990). Similarly, one may speculate that the immunocompromised state of AIDS patients, often coupled with poor nutritional status and extensive antibiotic therapy, may result in an altered intestinal microflora which then contributes to the increased susceptibility to *C. parvum* infection. In addition preliminary data from our laboratory suggest that the treatment of *C. parvum* infected AIDS patients with lactic acid producing bacteria may be of some benefit in alleviating symptoms (manuscript in preparation).

MODULATION OF GUT-ASSOCIATED LYMPHOID TISSUE BY LACTIC ACID PRODUCING BACTERIA.

A body of evidence indicates that physiological endogenous microflora has a key role to allow an appropriate development of mucosal immune system (*De Simone* et al., 1993). It has been suggested that this effect of microflora may be mediated by the adhesion of bacteria to gut-surface epithelium, which results in the active stimulation of GALT.

In this regard, we have shown that lactic acid producing bacteria are able to modulate several functions of GALT. In fact, we found that PP cell suspension cultures from BALB/c mice fed yoghurt containing living lactic acid producing bacteria exhibited a strong increase of blastogenic proliferative responses to mitogens such as phytohaemagglutinin (PHA) and LPS compared to controls (*De Simone* et al., 1993). Furthermore, the increased cell proliferation to LPS, which is mainly a mitogen for B lymphocytes, was correlated with an expansion of B lymphocyte pool in PP (*De Simone* et al., 1993). These latter data have been further supported by a recent report demonstrating that the content of immunoglobulin-synthesising cells in the jejunal lamina propria of germfree mice was significantly increased following oral and intraperitoneal administration of killed *L. acidophilus* strains (*Smeyanov* et al., 1992).

We observed results as comparable as in PP when the mitogen driven splenocyte proliferation was assayed. In fact, mice fed living lactic acid producing bacteria had a strong increase of splenocyte proliferation to lectins, including both mitogens for T cells (PHA,

ConA) and B cells (PWM) (*De Simone* et al., 1993). These effects were correlated with an expansion of T lymphocyte pool in the spleen (*De Simone* et al., 1993). In addition, we found that feeding lactic acid producing bacteria resulted in increased serum levels of IgM and IgG2a (*De Simone* et al., 1993). Furthermore, a brief treatment with heat proved sufficient to strongly reduce these effects of yoghurt, suggesting that its immunomodulating properties are strictly dependent on the presence of viable lactic acid producing bacteria (*De Simone* et al., 1993).

MODULATION OF MACROPHAGE FUNCTIONS BY LACTIC ACID PRODUCING BACTERIA

It is worth noting our finding in mice fed living lactic acid producing bacteria of increased production of oxygen metabolites by splenocytes following zymosan stimulation (*De Simone* et al., 1993; *De Simone* et al., 1988b) as well as the report of *Smeyanov* and coworkers (1992) showing that the oral and intraperitoneal administration of *L. acidophilus* to germfree mice lead to a significant rise in the level of luminol-dependent chemiluminescence of peritoneal macrophages. These data are in agreement with the hypothesis that lactic acid producing bacteria enhance both the phagocytic activity and the respiratory burst of monocyte-macrophage cells, probably resulting to enhance both phagocytosis and killing of pathogens. Moreover, the possibility that lactic acid producing bacteria upmodulate the functions of monocytes-macrophages as antigen-presenting cells should be considered. Further studies are required to better understand this issue.

MODULATION OF RESISTANCE TOWARD *SALMONELLA TYPHIMURIUM* BY LACTIC ACID PRODUCING BACTERIA

The demonstration that lactic acid producing bacteria are able to inhibit under *in vitro* conditions the growth of food-borne pathogens, including *Salmonella typhimurium*, (*Gilliland* and *Speck*, 1977) prompted to evaluate the mechanisms accounting for this antibacterial effect.

Natural antibiotics synthesised by lactic acid producing bacteria have been identified and, notably, bulgarican which is produced by *L. bulgaricus*, has been shown to possess a wide spectrum of *in vitro* antibacterial activity (*Reddy* et al., 1984). In addition, live microbial therapy has been shown in some reports to be more effective than the administration of antibiotics for treating infections from *Salmonella* spp. (*Hitchins* et al., 1985). However, these studies did not assay whether the protective effects of lactic acid producing against *Salmonella* infections are due to the enhancement of specific immune response.

Protection toward *Salmonella* is mediated in the early phase of infection by macrophages and by specific immunity in the late phase of infection (*Dichelte* et al., 1984; *Akeda* et al., 1981).

We have recently shown that yoghurt containing viable lactic acid producing bacteria strongly enhances murine defences against *S. typhimurium* via several mechanisms (*De Simone* et al., 1988c; *De Simone* et al., 1993), as

shown by:

a) increased antibacterial activity against *S. typhimurium* of PP mononuclear cells. Furthermore, this effect seems to be mediated, at least in part, by IgA antibodies. This increased activity is pivotal for inducing the host resistance toward invading salmonellae since their virulence is related to the ability to survive and multiply within PP microenvironment (*Dichelte* et al., 1984);
b) strongly increased absolute numbers of phagocytising macrophages, probably due to the accumulation of migratory macrophages from the pool of circulating monocytes at the sites of infection;
c) the strongly increased proliferative responses of splenocytes to both T cell and B cell mitogens, such as ConA and LPS.

Overall, these immunomodulating effects of administering viable lactic acid producing bacteria resulted in a strong reduction of *S. typhimurium* growth in both spleen and liver, therefore accounting for the higher survival rate of animals treated with viable lactic acid producing bacteria.

MODULATION OF AUTOIMMUNITY BY LACTIC ACID PRODUCING BACTERIA

The induction of coronary arteritis in mice by *L. casei* cell wall is thought to represent an animal model of the Kawasaki disease (*Tomita* et al., 1993). In fact, under *in vitro* conditions the treatment of vascular endothelial cells with supernatants from human PBMCs stimulated with *L. casei* cell wall has been shown to both enhance the adherence of polymorphonuclear cell (PMNs) to human endothelial cells and increase the expression of intercellular adhesion molecule-1 (ICAM-1) (*Tomita* et al., 1993). Notably, supernatants contained high concentrations of TNF-α and PMN adherence correlated directly with the concentration of TNF-α and both ICAM-1 expression and enhanced PMN adherence were inhibited by anti-TNF-α treatment (*Tomita* et al., 1993). In addition, the initial coronary inflammatory reaction in the mouse model has been shown to involve PMN adherence to vascular endothelium that has been activated by TNF-α released by PBMCs following stimulation with *L. casei* cell wall (*Tomita* et al., 1993).

LACTIC ACID PRODUCING BACTERIA IN THE TREATMENT OF DISEASES

Lactic acid producing bacteria are part of the normal Gram-positive anaerobic microflora. Through the production of lactic and acetic acids, hydrogen peroxide, and antimicrobial substances, such as bacteriocin-like compounds, these microorganisms contribute substantially to the maintenance of colonisation resistance, mostly against *Listeria monocytogenes*, *E. coli*, *S. typhimurium*, and *S. enteritidis* (*Fernandes* et al., 1988; *Lidbeck* and *Nord*, 1993; *Chateau* et al., 1993)). Therefore, it is considered important to maintain or increase the levels of lactic acid producing bacteria in the intestinal microflora to favourably alter the microecology of the gut and inhibit the growth of pathogenic bacteria. In fact, a body of evidence from both experimental and clinical

studies indicates that the administration of lactic acid producing bacteria could lead to significant changes in the intestinal microflora population (*Fernandes* et al., 1988; *Johansson* et al., 1993; *Kafarskaya* et al., 1993). However, some reports suggest that lactic acid producing bacteria should be taken continuously in order to maintain high levels of these protective bacteria in the small intestine (*Lidbeck* et al., 1987).

Furthermore, it is well known that disturbances in the normal intestinal microflora leads to gastrointestinal disorders often resulting in diarrhoea (*Fernandes* et al., 1988; *Johansson* et al., 1993). In addition, the colonisation of the gastrointestinal tract by foodborne pathogenic bacteria is as a rule correlated with a strong decrease in the counts of endogenous intestinal lactic acid producing bacteria (*Fernandes* et al., 1988; *Johansson* et al., 1993)

The ability of lactic acid producing bacteria to affect both systemic and mucosa-associated immune response, as reported above, suggests that the administration of lactic acid producing bacteria could strongly contribute to promote the recovery from infections. For example, *Lactobacillus* spp. strain GG has been recently shown to promote the recovery of children with rotavirus diarrhoea via augmenting both local and systemic immune defence (*Kaila* et al., 1992). Furthermore, specific IgA response has been endorsed, which is probably relevant in allowing protection against rotavirus reinfections (*Kaila* et al., 1992). However, a systemic immune response to lactic acid producing bacteria has been demonstrated to be triggered by their interaction with intestinal mucosa (*Takahashi* et al., 1993). Whether this phenomenon could be detrimental for the therapeutic efficacy of administering lactic acid producing bacteria to patients with intestinal infections, such as rotavirus diarrhoea, remains to be established.

Currently available literature suggests that, in addition to the prophylaxis of intestinal and urogenital infections, potential beneficial effects of lactic acid bacteria include the prophylaxis and the treatment of lactose maldigestion, cholesterol metabolism, and diarrhoeal disorders (*Marteau* et al., 1993).

Finally, lactic acid producing bacteria could have a role in the surveillance against tumours, as suggested by recent experiments assaying urinary mutagenicity in humans (*Hayatsu* et al., 1993). The effect of 3-week oral administration of *Lactobacillus casei*, which is commonly found in yoghurt, on the urinary mutagenicity derived from the ingestion of fried ground beef has been evaluated. The comparison of the urinary mutagenicity found before and after the *L. casei* treatment showed that the treatment resulted in a strong decrease of mutagenicity (*Hayatsu* et al., 1993). This suppressing effect is likely to be related to the changes in the intestinal microflora population induced by *L. casei* supplementation. Further studies are required to evaluate the possible role of administering lactic acid producing bacteria in the prevention of malignancy.

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