SYMPOSIUM: PROBIOTIC BACTERIA FOR HUMANS: CLINICAL SYSTEMS FOR EVALUATION OF EFFECTIVENESS

Immune System Stimulation by Probiotics

G. PERDIGON,[1,2] S. ALVAREZ,[1,2] M RACHID,[2] G. AGÜERO,[1,2] and N. GOBBATO[2]
Centro de Referencias para Lactobacilos (CERELA)
Chacabuco 145
4000 Tucumán, Argentina
and
Instituto de Microbiología
Facultad de Bioquímica, Química y Farmacia
Universidad Nacional de Tucumán
Ayacucho
492 Tucumán, Argentina

ABSTRACT

The immune system consists of organs and several cell types. Antigen interaction with these cells induces a cellular immune response mediated by activated cells and a humoral immune response mediated by antibodies. The cellular interactions are enhanced by adhesion molecules, and the activated cells release different cytokines. These complex cellular interactions induce a systemic immune response. If the antigen penetrates by the oral route, a secretory immune response is obtained, which is mediated by secretory IgA. The determination of the number of T or B cells, the quantitative or qualitative measure of the cytokines, antibody levels, or the study of cellular function such as phagocytic activity is used to evaluate the state of the immune system.

The effects of lactic acid bacteria on the systemic immune response and on the secretory immune system are described. Potential health benefits of lactic acid bacteria include protection against enteric infections, use as an oral adjuvant, the immunopotentiator in malnutrition, and the prevention of chemically induced tumors. The results showed that *Lactobacillus casei* could prevent enteric infections and stimulate secretory IgA in malnourished animals, but could produce bacteria translocation. Yogurt could inhibit the growth of intestinal carcinoma through increased activity of IgA, T cells, and macrophages. (**Key words**: immune system, probiotic, immunopotentiation, cytokines)

Abbreviation key: **BL** = B lymphocytes, **HLA** = human leukocyte antigens, **IL** = interleukin, **LPS** = lipopolysaccharide, **PMN** = polymorphonuclear, **sIgA** = secretory IgA, **TH** = T helper, **TL** = T lymphocytes.

INTRODUCTION

The immune system of vertebrates consists of a number of organs and several different cell types that recognize accurately and specifically foreign antigens on microorganisms and, thereby, eliminate those organisms.

The organs of the immune system are bone marrow, thymus, spleen, Peyer's patches, and lymph nodes. The cells of this system are the leukocytes, or white blood cells. There are two categories of leukocytes: 1) phagocytes, including neutrophils, monocytes, and macrophages, which form part of the innate immune system and provide nonspecific immunity, and 2) the lymphocytes, which mediate adaptative, or specific, immunity.

The two basic kinds of phagocytes are mononucleated cells, such as monocytes and macrophages, and polymorphonuclear granulocytes, such as the neutrophils, basophils, and eosinophils.

The two types of lymphocytes have different functions. The T cells are differentiated in the thymus in different subpopulations, the T

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[1]Centro de Referencias para Lactobacilos.
[2]Instituto de Microbiologia.

helper (TH) or T cytotoxic-suppressor cells, with different markers to identify them: CD4+ for TH and CD8+ for T cytotoxic or suppressor cells (15). The TH cells have three subtypes, TH1, TH2, and TH0, which are distinguished functionally by the product of their secretions (7).

The B cells are differentiated in the bone marrow and consist of two populations; the first constitutes 15% of the population, is identified by a characteristic marker, CD5+, and is found in the peritoneal cavity. The other population constitutes 85% of B cells and is found in the blood and all organs of the immune system (17). There is also a population of "null" cells, non-T non-B cells, and their differentiation is uncertain. Those cells, the killer (K) and natural killer (NK) cells, have important functions in host defenses against tumors.

When a foreign antigen penetrates the body, the cellular interaction of the immune system is produced. This interaction can induce an immune response that is specific, nonspecific, or both. The first response against the antigen is the nonspecific immune response through an inflammatory response in which the phagocytic cells actively participate.

The inflammatory response is characterized by great cellular infiltration, which releases inflammatory mediators (3, 16). If enhanced, this response can damage the tissue. The first step in the inflammatory response is characterized by the appearance of neutrophils at the lesion site that fulfill their phagocytic function and release chemotactic substances to attract the macrophages that participate in the second step. Macrophages have a phagocytic function similar to that of neutrophils, but, once the antigen is degraded, some peptides of this antigen can be expressed on the macrophage membrane. Therefore, the macrophage can present the antigen to the lymphocytes, which participate in the specific immune response. This presentation is realized through the association of the antigen with the special molecules that are present on their surface: histocompatibility antigens (1). There are two kinds of human leukocyte antigens (HLA): HLA class I are expressed in all cells of the body, and HLA class II are expressed in the immune cells and some nonimmune cells, such as epithelial cells (9).

The specific immune response can produce cellular immunity mediated by specifically sensitive immune cells and the humoral immune response mediated by the antibody production. Cellular immunity has great importance in the host defense against tumors. Induction of an immune response needs not only cellular interaction but also cytokines and adhesion molecules. The cytokines are substances produced by immune or nonimmune cells and activated by the antigen stimulation (6). In the cytokines are the interleukins (IL). Currently, more than 11 IL are known; these IL are produced by different kinds of TH lymphocytes, macrophages, endothelial cells, epithelial cells, and fibroblast cells. Other cytokines are the growing cellular factors, such as granulocyte colony-stimulating factor; macrophage colony-stimulating factor; fibroblast growth factor; substances that promote the cellular differentiation of transforming growth factor; interferon-α, -β, or -γ; tumor necrotic factor; and metabolites of arachidonic acid, such as leukotrienes and prostaglandins E_1, D_2, and I_2 (13).

All cytokines display multiple activities, and all immune cells selectively express specific membrane receptors for some of them, allowing the precise and intimate interaction of various cell types in a process that is now recognized as the "cytokine network" (4).

The adhesion molecules between cells (18) are the second signal to obtain an immune response. Termed integrins or selectins, these molecules are intercellular adhesion molecule, leukocyte function-associated antigen (LAF_1 and LAF_2), and very late antigen (VLA_1 and VLA_2).

SYSTEMIC IMMUNE RESPONSE

When the antigen penetrates the body parenterally, a systemic immune response is produced.

1. The nonspecific immune response occurs through the inflammatory response with the active participation of the phagocytic cells [polymorphonuclear (PMN) and macrophage].
2. The processed antigen is expressed in the membrane of the antigen-presenting cell and shown to the lymphocytes through

the HLA class I or class II pathway. The HLA class I molecules generally present cytoplasmic antigens (endogenous antigens) that are primarily synthesized by the infected cells, for example, viral proteins. In contrast, general HLA class II molecules primarily present peptides derived from exogenous antigens, for example, those internalized by the antigen-presenting cells from the extracellular medium [e.g., soluble protein antigen, or antigen derived from microorganisms (8)].

3. In the specific immune response, if the antigen is associated to HLA class I, a cytotoxic cellular response is obtained through cytotoxic T lymphocyte (**TL**), which has the marker CD8+. If the antigen is associated to HLA class II, a humoral response with antibodies is obtained through the TL helper CD4+. This last population cooperates with B lymphocytes (**BL**), which become plasma cells, producing any of the five classes of Ig (IgM, IgG, IgA, IgE, and IgD). Through these cellular interactions, the immune response is increased or abolished by the cytokine action.

SECRETORY IMMUNE RESPONSE

If the antigen penetrates by oral route, the first immune response that occurs is oral tolerance, through the intraepithelial lymphocytes that carry the CD8+ marker. However, oral tolerance can be abrogated, and then an immune response is produced. The stimulation of one response or the other depends on the physical state of the antigen; thus, in general, soluble antigens induce oral tolerance, and particulated antigens produce an immune response at the mucosal level. This immune response is mainly a humoral immune response; the number of IgA-producing cells and the secretory IgA (**sIgA**) synthesis increase. Although all of the immune cells are present at the mucosa, the cytotoxic cellular response at the mucosa is limited by immunoregulatory mechanisms to avoid intestinal damage.

The events of the secretory immune response are the following. Luminal antigen is transported into Peyer's patch through M cells of the follicle-associated epithelium and presented to T cells by HLA class II dendritic cells or macrophages. Antigen is presented by T cells to B cells. Primed T and B cells migrate through to the peripheral blood circulation and extravasation mainly in the gut lamina propria and in other exocrine tissues. Intestinal B cells are differentiated to plasma cells producing IgA and CD8+ T cells that migrate into the epithelium to mediate oral tolerance to food antigens (5).

IMMUNE RESPONSE INDUCED BY LACTIC ACID BACTERIA

To study the effect of lactic acid bacteria on the immune system, the oral route, which is the natural host route of these bacteria, should be emphasized.

When the antigen gets into the body by an oral route, the systemic immune stimulation is produced by the cytokines released by lymphoid cells associated with the mucosa, which interact with the antigen. This response may be measured through in vitro assays by determining the cellular activity (e.g., the phagocytic activity) or the products released during the cellular interactions; examples are cytokines, using special cellular lines, such as the mono Mac 6 human macrophage cell line; human epithelioid carcinoma, cell transfected with cDNA for IL4 and IL5; and Caco-2 human colon carcinoma cell line. Then, the systemic immune response may be determined by measuring the nonspecific immune response through phagocytic activity of the peritoneal macrophages, which were stimulated by interleukins, and the specific immune response may be evaluated by measuring the activation grade of TL by assays of delayed-type hypersensitivity or by antibody level increased from TL and BL activation through cytokines.

However, great activation of the immune system in a healthy host cannot be conveniently obtained, because the immune system is always in equilibrium with other systems (e.g., the nervous and endocrine systems); a general activation of the immune system by constant antigen stimulation could produce negative effects on the host, including autoimmunity. A circumstance that would require immune system stimulation is primarily immunosuppression from antitumor therapies, but never when this immunosuppression is caused by a therapy for autoimmune illness.

Lactic acid bacteria might benefit the host in some situations, such as to prevent enteric infections or to act as immunomodulatory agents in other processes. The therapeutic use

of lactic acid bacteria must consider the effects of these bacteria on the intestinal microenvironment, especially the microflora, which are responsible for oral tolerance.

It is absolutely necessary to know the mechanism by which the lactic acid bacteria and the fermented product stimulate the immune system and to be aware of the effect of the biologically active peptides produced in the fermentation process on the immune cells.

The study of these mechanisms involves the use of experimental animals, most importantly the mouse, as a model to study the behavior of the immune system; most of these results can then be extrapolated to human beings. Thus, the first studies of histocompatibility antigen and T cells used mice. When these studies were conducted using human beings, the patterns obtained were identical to those for mice. In addition, the animal models provide an understanding of the biology of different tumors.

The effect of yogurt and some lactic acid bacteria on the systemic immune response and mucosal immunity has been studied using Albino Swiss and BALB/c mice as experimental models.

EFFECT OF LACTIC ACID BACTERIA AND YOGURT ON THE SYSTEMIC IMMUNE RESPONSE

We studied the effect on the systemic immune response of the following lactic acid bacteria: *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus delbrueckii* ssp. *bulgaricus, Streptococcus thermophilus,* and yogurt. The nonspecific immune response was measured on peritoneal macrophages by phagocytic assays and by the colloidal carbon clearance test. By this latter method, we determined the activity of the mononucleated phagocytic system.

The specific immune response was measured by plaque-forming cells, concentrations of circulating antibodies, and delayed-type hypersensitivity test using the sheep red blood cells as the antigen. These methods elucidated the activity of TL and BL.

According to our results, we concluded that *L. acidophilus, L. casei, L. delbrueckii* ssp. *bulgaricus,* and yogurt induced increased systemic immune response at different levels of stimulation and also that *L. casei* was the most effective (11).

EFFECT OF LACTIC ACID BACTERIA ON THE SECRETORY IMMUNE SYSTEM

We also studied the effect of lactic acid bacteria and yogurt on the secretory immune system. The number of IgA-producing cells was determined by immunofluorescent test on histological slices of the small intestine; concentrations of sIgA were measured in the intestinal fluid by ELISA (2).

The mice were fed with yogurt and the different lactic acid bacteria at a rate of 1.2×10^9 cells/d per mouse during 2, 5, and 7 consecutive d. The results showed (Figure 1) that *L. casei, L. acidophilus,* and yogurt enhanced the number of IgA-producing cells and that this effect increased as the dose increased. We observed that, during 7 d of feeding with *L. acidophilus,* the number of IgA-producing cells decreased. We made histological slices of the small intestine to observe whether or not the total cellular population decreased in the lamina propria of the intestine. Long-term administration of *L. acidophilus* decreased the total number of lymphoid cells. We also found alterations of the epithelium that did not occur when *L. casei* or yogurt was administered.

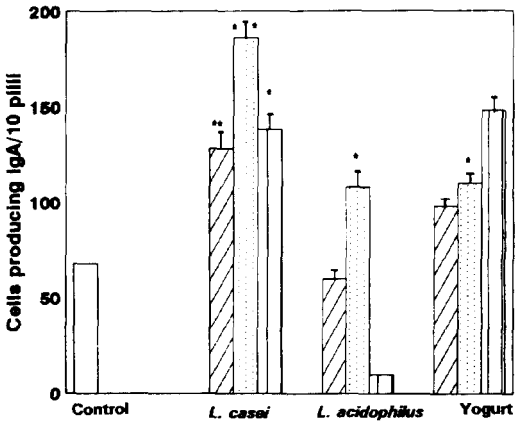

Figure 1. Effect of dietary *Lactobacillus casei, Lactobacillus acidophilus,* and yogurt for 2, 5, and 7 consecutive d on the number of cells producing IgA in the small intestine of mice as determined by immunofluorescence test. Values are means (n = 3); error bars are standard deviations. Differences were significant at 2, 5, and 7 d of feeding with *L. casei,* 5 d for *L. acidophilus,* and 5 and 7 d for yogurt (*$P < .05$; **$P < .01$). Untreated control mice (open bar), mice fed for 2 d (diagonally striped bar), mice fed for 5 d (dotted bar), and mice fed for 7 d (vertically striped bar).

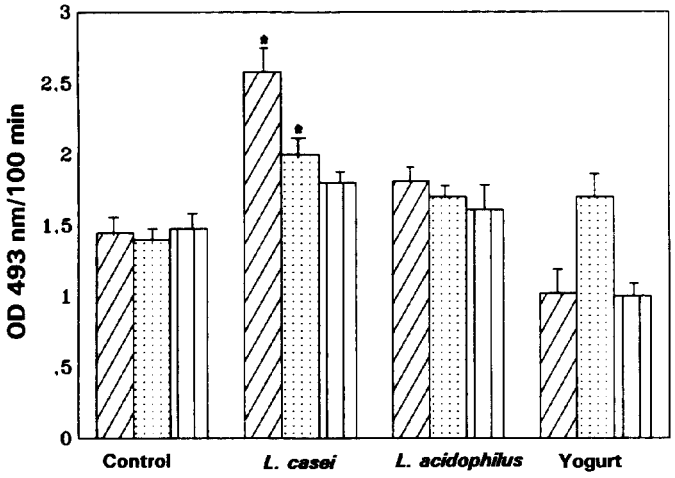

Figure 2. Concentrations of IgA against *Salmonella* in intestinal fluid of the mice fed with *Lactobacillus casei* or *Lactobacillus acidophilus* for 2 d and with yogurt for 7 consecutive d. At the end of each feeding period, the mice were challenged with *Salmonella typhimurium*. The IgA concentrations were measured by ELISA on d 2, 5, and 7 postchallenge. Controls are untreated mice. Values are means (n = 5); error bars are standard deviations. The IgA concentrations 2 d postchallenge (diagonally striped bar), IgA concentrations 5 d postchallenge (dotted bar), and IgA concentrations 7 d postchallenge (vertically striped bar). Asterisks indicate significant difference ($P < .05$).

When sIgA that were specific for *S. typhimurium* antigen were measured on different days postchallenge, sIgA concentrations were dose-dependent (Figure 2). The optimal dose (2.4×10^9 cells) for *L. casei* and *L. acidophilus* was after 2 d of feeding and, for yogurt, 7 d of feeding.

Then, TL were determined on histological slices of the small intestine by immunofluorescent test for 2 d of feeding *L. casei* and different doses of yogurt. The results showed (Figure 3) a significant augmentation of the number of TL for *L. casei* and yogurt, the effect of yogurt being dose-dependent (our unpublished results). This increase of TL is very important because the T cells have an important role on the mucosal immune response; therefore, the number of sIgA cells are enhanced through the cytokines, which are released by TL (14).

From these results, we conclude that *L. casei* and yogurt increase the mucosal immunity, depending on the dose, without the side effects at the intestinal level that had been observed in the histological slices. How can this knowledge be applied? In what circumstances could lactic acid bacteria and yogurt be used as immunomodulatory agents and under what conditions? Bearing in mind that the lactic acid bacteria and yogurt stimulate the sIgA, which is very important in the defense against enteropathogens, could these substances be used in the prevention of enteric infections? Could the lactic acid bacteria be used as an oral adjuvant of vaccines? If they can act as adjuvants of the immune system, what is their action against immunosuppression in tumor growth? To answer these questions, we studied 1) the preventive effect of *L. casei, L. acidophilus,* and yogurt against *S. typhimurium* infection; 2) the perspective of the use of *L. casei* as oral adjuvant of vaccine; 3) the effect of *L. casei* on a model of immunosuppression by malnutrition; and 4) the action of yogurt on an intestinal tumor that was chemically induced.

PREVENTIVE EFFECT OF *L. CASEI, L. ACIDOPHILUS,* AND YOGURT AGAINST *S. TYPHIMURIUM* INFECTION

Mice were fed with the lactic acid bacteria and yogurt during 2, 5, and 7 consecutive d. At the end of each feeding period, mice were challenged with a dose (10^6 cells) of *S.*

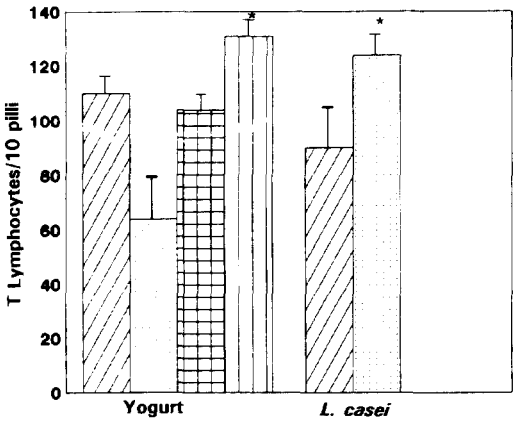

Figure 3. Effect of diets with yogurt for 2, 5, and 7 consecutive d and with *Lactobacillus casei* for 2 d on the population of T lymphocytes in the small intestine, as determined on histological slices from small intestine by an immunofluorescence test. Controls are untreated mice. Values are means (n = 3); error bars are standard deviations. Asterisk indicates significance ($P < .05$). Untreated control mice (diagonally striped bars), mice fed for 2 d with yogurt or *L. casei* (dotted bars), mice fed for 5 d with yogurt (checked grid bars), and mice fed for 7 d with yogurt (vertically striped bars).

typhimurium at 20 times the median lethal dose. The preventive effect of this feeding was measured by colonization assays of the liver and spleen and by the specific IgA that were present in the intestinal fluid.

The results showed that *L. acidophilus* did not prevent against *S. typhimurium* infection. The prevention with *L. casei* was total with 2 d of feeding, partial with 7 d of feeding, and none with 5 d of feeding. We cannot explain why the 5-d dose was not effective, but we think that the autoregulatory mechanisms may have occurred. Yogurt partially prevented the *S. typhimurium* infection; the 7-d dose was the most effective. All of these results were closely related to IgA concentration, as determined from tests of the intestinal fluid. That is, major protection was afforded when the sIgA concentrations increased. Thus, we concluded that *L. casei* was the most effective in the prevention of an infection against *S. typhimurium* (12).

PERSPECTIVE OF THE USE OF *L. CASEI* AS AN ORAL ADJUVANT OF VACCINE

Because we showed that *L. casei* (2.4×10^9 cells) administered during 2 consecutive d effectively stimulated mucosal immunity, which protected from enteric infections, and that this effect depends on cellular viability, we think that *L. casei* could be an excellent oral adjuvant to an enteric vaccine. This possibility is important because very few adjuvant substances can be used in human beings. This experiment determined the duration of the stimulation of the secretory immune system from *L. casei* and, thus, the interval between doses. Adjuvant capacity of *L. casei* was compared with that of lipopolysaccharide (LPS).

We also measured the adjuvant activity of *L. casei* that was associated with LPS because LPS substance is the main structure of the cellular wall of the enteropathogen. This mixture may be effective when it is used in oral vaccines against enteric infection.

The mice were fed for 2 d with *L. casei*, LPS, or a mixture of *L. casei* plus LPS (4 μg). Figure 4 shows that *L. casei* plus LPS increased the response to the antigen LPS with high concentrations of sIgA until the 7-d postadministration of *L. casei* plus LPS. If the mice received a booster dose on d 15 postpriming (Figure 5), this mixture protected effectively against *S. typhimurium* infection. Increases of sIgA, but not IgM, concentrations were observed (Figure 6). This increase of IgA is important because IgA helps to control the infection. An increase of IgM would mean that the stimulus did not induce memory cells. The presence of memory cells is important to maintain good secretory immune response. At this time, different populations of TL are being studied to verify the hypothesis mentioned.

EFFECT OF *L. CASEI* ON A MODEL OF IMMUNOSUPPRESSION BY MALNUTRITION

We studied whether *L. casei*, which induced an increase of sIgA in well-nourished mice, could be used to treat cases of malnutrition, which cause a great number of deaths from enteric infections. Mice that had been malnourished by protein deprivation were 1) treated with milk at 10% (vol/vol) or 2) treated with a suspension of *L. casei* (1.2×10^9 cells) in milk (10%, vol/vol) for 2 consecutive d. At the end of these treatments, we studied the number of cells producing IgA, the concentrations of the sIgA and IgM, and the variations

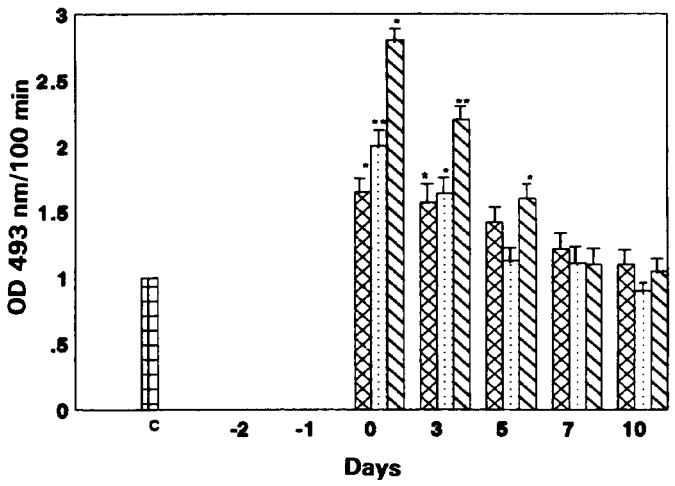

Figure 4. Adjuvant effect of *Lactobacillus casei* as measured by the IgA concentrations in the intestinal fluid by ELISA test for mice treated with *L. casei,* lipopolysaccharides (LPS), or the mixture of the *L. casei* plus LPS. The mice were treated for 2 d (–2, –1), and the ELISA test was performed on d 3, 5, 7, and 10 posttreatment. Time 0 indicates IgA concentrations immediately after administration. Values are means (n = 5); error bars are standard deviations. Untreated control mice (checked grid bars), viable *L. casei* (crosshatched bars), LPS (dotted bars), and mixture of the *L. casei* plus LPS (diagonally striped bars).

in the intestinal flora. The milk at 10% plus the *L. casei* suspension induced an increase in the number of cells producing IgA in relation to those in the untreated, malnourished controls (Table 1). The IgA concentrations were slightly higher, and IgM concentrations were significantly higher, than those of controls, but the infection by *S. typhimurium* was not con-

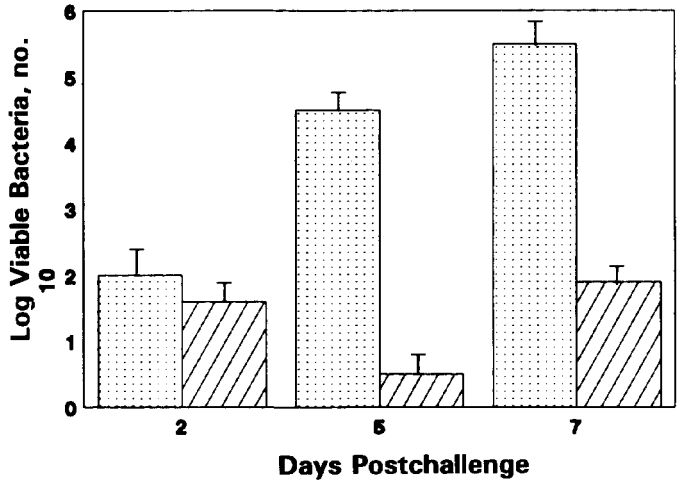

Figure 5. Effect of boosting with *Lactobacillus casei* plus lipopolysaccharides (LPS) on d 15 postpriming on protection against *Salmonella* infection. Mice were treated with *L. casei* plus LPS for 2 d, a single-dose booster on d 15 postpriming, and challenge with *Salmonella typhimurium.* Viable bacteria in liver and spleen were determined on 2, 5, and 7 d postinfection. Values are means (n = 5); error bars are standard deviations. Control mice without treatment (dotted bar); mice treated, boosted, and challenged (diagonally striped bar).

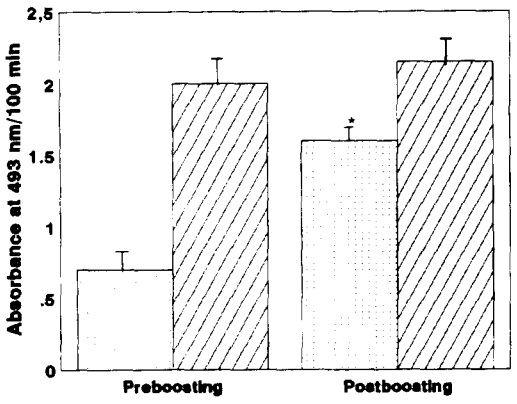

Figure 6. Concentrations of IgA and IgM before and after boosting with a single dose of the *Lactobacillus casei* plus lipopolysaccharides (LPS) on d 15 postpriming. The Ig were measured in the intestinal fluid of mice by an ELISA test. Difference between IgA concentrations were significant (*$P < .05$). Values are means (n = 5); error bars are standard deviations. Concentrations of IgA (dotted bar) and IgM (diagonally striped bar).

trolled in any case. When intestinal flora were studied, *L. casei* administration improved the intestinal flora qualitatively and quantitatively (Figure 7) (Perdigón et al., 1994, unpublished data). However, the translocation of the microorganisms of the normal flora in the liver was observed. We are currently studying, using electronic microscopy, the modifications of the intestinal epithelium that are induced by *L. casei*. *Lactobacillus casei* induces translocation of bacteria in malnourished mice; thus, *L. casei* could be used as adjuvant during malnutrition, but only after refeeding.

EFFECT OF YOGURT ON CHEMICALLY INDUCED INTESTINAL TUMORS

For our study, mice were fed with yogurt during 2, 5, 7, or 10 consecutive d. At the end of each feeding period, an intestinal tumor was induced with 1-2-dimethylhydrazine that was administered intramuscularly. After induction, after 10 d, and after 5 mo, yogurt feeding at the mentioned doses was repeated cyclically.

Inhibition of tumor growth was determined through histological examination. The cells producing IgA, the TL, were characterized by immunofluorescence test, and the macrophages by a phagocytosis in vivo assay using the dextran-iron technique.

The results showed that yogurt fed for 7 and 10 d inhibited the development of the intestinal carcinoma; only a lymphoid infiltration was observed, and the untreated controls showed atypical cells and development of the tumor. The IgA-producing cells and TL also increased in the large intestine during the different feed-

TABLE 1. Effect of *Lactobacillus casei* feeding of malnourished mice.

	Time after infection	Control[2]	Control fed with *L. casei* for 2 d	Malnourished[1]		
				Control[3]	Fed with 10% milk for 2 d	Fed with *L. casei* for 2 d
	(d)					
Bacteria,[4] $\log_{10}$, no.	2	1.0	.0	3.9	1.1	0
	5	3.9	.8	4.1	4.0	4.0
	7	3.1	.5	3.8	3.7	4.0
IgA, 493 nm/100 min	2	.8	2.5	.5	.60	.40
	5	.6	2.3	.4	.64	.80
	7	.97	1.3	.4	.52	.50
IgM, 493 nm/100 min	2	3.0	3.0	1.3	.80	.50
	5	2.87	3.55	1.13	2.30	2.3
	7	2.20	3.12	.99	2.20	2.2
IgA Cells, no.	0	100	120	55	98	110

[1]Mice were malnourished by protein deprivation at weaning without treatment with *L. casei*.

[2]Well-nourished mice without treatment with *L. casei*.

[3]Malnourished mice without treatment with either milk or *L. casei*.

[4]*Salmonella typhimurium* isolated from liver and spleen in the colonization assays.

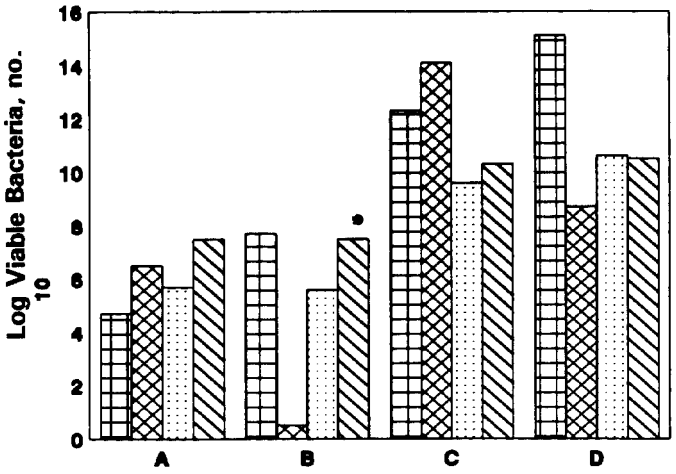

Figure 7. Influence of diet containing milk (10%, vol/vol) or milk (10%, vol/vol) plus *Lactobacillus casei* for 2 d on the facultative and strict anaerobes in the small and large intestine of malnourished mice. A = Small intestine, facultative anaerobes; B = small intestine, strict anaerobes; C = large intestine, facultative anaerobes; D = large intestine, strict anaerobes. Values are means (n = 5); error bars are standard deviations. Asterisk indicates significant difference (*P < .01). Microflora of well-nourished mice (checked grid bars), microflora of malnourished mice without treatment with *L. casei* (crosshatched bars), microflora of malnourished mice fed with milk (dotted bars), and microflora of malnourished mice fed milk plus *L. casei* (diagonally striped bars).

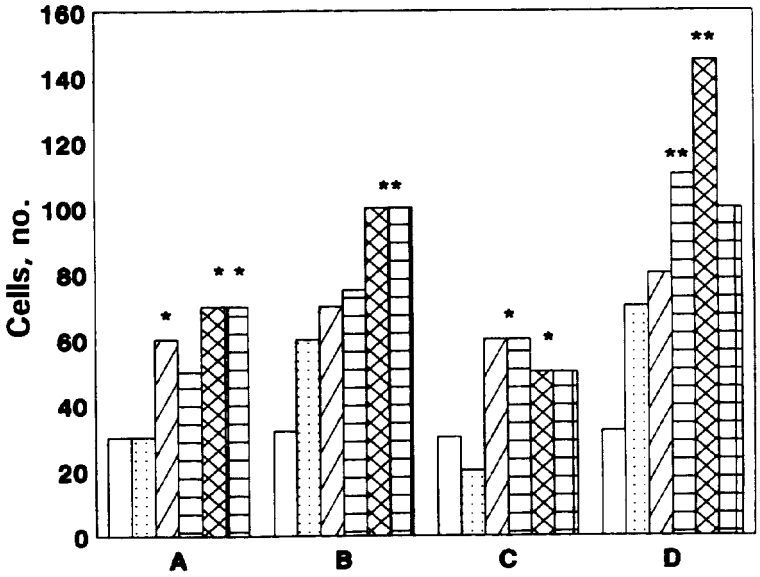

Figure 8. Effect of diets containing yogurt for 2, 5, 7, and 10 consecutive d on cells producing IgA and on T lymphocytes (TL) in the large intestine of mice. Mice were treated with a carcinogen 1-2-dimethylhydrazine (DMH) and with DMH fed cyclically with different doses of yogurt. Cells producing IgA and TL were determined by an immunofluorescence test on histological slices after 2 and 4 mo of treatment. Differences were significant for cells producing IgA and for TL during all periods of yogurt feeding (*P < .05; **P < .01). Values are means (n = 5); error bars are standard deviations. A = 2-mo treatment, IgA; B = 2-mo treatment, TL; C = 4-mo treatment, IgA; and D = 4-mo treatment, TL. Control mice without treatment (open bars), mice treated with DMH (dotted bars), mice treated with DMH yogurt 2 d (diagonally striped bars), mice treated with DMH yogurt 5 d (horizontally striped bars), mice treated with DMH and yogurt for 7 d (cross hatched bars), and mice treated with DMH and yogurt for 10 d (checked grid bars).

ing periods (Figure 8). When the activation of macrophage in the large intestine was analyzed, activation was great for the treated, but not for the untreated, mice (unpublished results).

The increase of IgA may indicate that the mechanisms by which the yogurt inhibits tumor development could be through the decrease of inflammatory response.

CONCLUSIONS

Our results showed that *L. casei* could prevent enteric infections but that the effect was dose-dependent.

Also, *L. casei* could be used as adjuvant in oral vaccine. *Lactobacillus casei* stimulated IgA and IgM synthesis in malnourished mice, but to avoid translocation use is not advised before refeeding. Yogurt could inhibit the growth of intestinal carcinoma, increasing the number of IgA and TL and the activity of intestinal macrophages.

Although these results were obtained using mice as experimental models, they are not easily transferred to human beings. We think that, for the use of lactic acid bacteria as immunomodulatory agents, experimental models are absolutely necessary to determine that effects of lactic acid bacteria on the host are innocuous and to select those bacteria that most effectively enhance the immune response. However, the exploitation of that knowledge for therapeutic purposes is still limited.

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