BIOCELL
2000, 24(3): 223-232

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Gut Mucosal Immunostimulation by Lactic Acid Bacteria

E. VITIÑI[1], S. ALVAREZ[1,2], M. MEDINA[2], M. MEDICI[2], M. V. DE BUDEGUER[3] AND G. PERDIGÓN[1,2]

1- Cátedra Inmunología, Instituto de Microbiología. Facultad de Bioquímica, Química y Farmacia.Universidad Nacional de Tucumán.
2- Centro de Referencias para Lactobacilos.CERELA.Chacabuco 145. 4000 Tucumán. Argentina.
3- Cátedra de Histología. Facultad de Medicina. UNT.

Key words: mucosal immune cells, intestine, Lactic Acid Bacteria

ABSTRACT: The beneficial properties of lactic acid bacteria (LAB) on human health have been frecuently demonstrated. The interaction of LAB with the lymphoid cells associated to the gut to activate the mucosal immune system and the mechanisms by which they can exert an adjuvant effect is still unclear, as well as if this property is common for all the LAB. We studied the influence of the oral administration of different genous of LAB such as *Lactobacillus casei*, *L. acidophilus*, *L. rhamnosus*, *L. delbrueckii* subsp. *bulgaricus*, *L. plantarum*, *Lactococcus lactis* and *Streptococcus thermophilus*. We determined if the LAB assayed were able to stimulate the specific, the non-specific immune response (inflammatory response), or both. We demonstrated that all the bacteria assayed were able to increase the number of IgA producing cells associated to the lamina propria of small intestine. This effect was dose dependent. The increase in IgA^+ producing cells was not always correlated with an increase in the $CD4^+$ T cell number, indicating that some LAB assayed only induced clonal expansion of B cells triggered to produce IgA. Most of them, induced an increase in the number of cells involved in the inflammatory immune response. $CD8^+$ T cell were diminished or not affected, with exception of *L. plantarum* that induced an increase at low dose. This fact would mean that LAB are unable to induce cytoxicity mechanisms.

We demonstrated the importance in the selection of LAB to be used as gut mucosal adjuvant. The different behaviours observed among them on the gut mucosal immune response, specially those that induce inflammatory immune response, show that not all the LAB can be used as oral adjuvant and that the beneficial effect of them can not generalized to genous or specie. The immunoadjuvant capacity would be a property of the strain assayed.

Introduction

The intestinal microenvironment is extremely complex. It contains normal microflora and immune cells associated to mucosa surfaces. The indigenous microflora of the digestive tract consist of autochthonous microorganisms which largely stay in the host and the transient one. These microorganisms play a role in the development and maintainance of the activity of the immune system associated to the gut-associated-lymphoid tissue (GALT); that includes IgA, $CD4^+$, $CD8^+$ T cells and intraepithelial lymphocyte (IEL) activation. It has been demonstrated that obligate anaerobic Gram positive bacteria not only provide the stimulus for populating the lamina propria with IgA cells but also for the increase of cellularity in the intraepithelial compartment (Moreau *et al.*, 1978; Klasen *et al.*, 1993). It was suggested that the gut flora can be modified by the ingestion of certain non pathogenic microorganisms called

Address correspondence to: Dra. Gabriela Perdigón, CERELA, Chacabuco 145, (4000) San Miguel de Tucumán, ARGENTINA. Fax: (+54-381)4310465. E-mail: perdigon@cerela.org.ar
Received on March 23, 2000. Accepted on August 3, 2000.

"probiotics" (Fuller, 1992). A group of these bacteria are the lactic acid bacteria (LAB), which can influence the intestinal microenvironment and to produce beneficial effects in the host.

Lactic acid bacteria and the fermented products containing these microorganisms can decrease blood cholesterol level, induce antitumour immunity and secretory immune system stimulation (Agerback *et al.*, 1995; Matzusaki *et al.*, 1985; Schiffrin *et al.*, 1995; Sanders, 1993; Perdigón *et al.*, 1998).

The use of LAB fermented milks as oral adjuvant has been suggested in the prevention against enteric infection (Saavedra *et al.*, 1994; Isolauri *et al.*, 1991). However, its use in human being is still limited, because there is none or poor information about the mechanisms involved to exert beneficial effects on the host (Halmiton-Miller, 1996).

Although direct transposition of results from animals studies to human, is not always possible, they may serve to give an indication of the potential benefits available of these probiotic bacteria and also give information about how the probiotics work, and what immune cells are activated.

In previous studies we have demonstrated that different LAB are able to activate the systemic immune response (Perdigón *et al.*, 1986, 1988) and that *L. casei* was able to induce a secretory immune response depending of the dose administered (Perdigón *et al.*, 1991, 1995).

The aim of this work, was to study how the ingestion of LAB as mucosal adjuvant can influence on the activation of the immune cells associated to the gut, and if the oral immunoadjuvant property described for the LAB assayed can be extrapolated to the genous or specie.

Materials and Methods

Animals

Female BALB/c mice weighing 25-28 g of 6 weeks age were obtained the random-bred colony kept in our department at the Institute of Microbiolgy. Each experimental group consisted of 3 mice/day of LAB administration. Each assay was performed by duplicate or triplicate.

Microorganisms

The bacterial strains used for experiments were: *L. casei* CRL 431, *L. acidophilus* CRL 924, *Lac.lactis* CRL 526, *L. plantarum* CRL 936, *L. delbrueckii* subsp. *bulgaricus* CRL 423, *L. rhamnosus* CRL 74 and *S. thermophilus* CRL 412, from CERELA culture collection.

L. casei, *L. acidophilus*, *L. rhamnosus*, *L. plantarum* and *L. delbrueckii* ssp. *bulgaricus* were cultured for 8 h at 37°C in MRS (De Man *et al.*, 1960) broth (Oxoid Ltd, U.S.A); *S. thermophilus* and *Lac. lactis* were cultured 8 h at 37°C and 30°C respectively in LAPTg (Lactose, peptone, tryptone-glucose; Raibaud *et al.*, 1961) broth. All of them were harvested by centrifugation at 5,000 g for 10 min, and washed three times with sterile saline solution.

Feeding procedure

Mice were fed daily with each culture of LAB at 10^9 CFU/day/animal for 2, 5 or 7 consecutive days. The cultures, suspended in sterile 10% non-fat milk (NFM) were administered at 20% v/v in the drinking water. The control group received sterile milk (NFM) 10% in the drinking water, given under the same conditions as those used for the test groups.

Immunofluorescence test

The number of IgA-secreting cells, $CD4^+$ and $CD8^+$ T lymphocytes were determined on samples from the ileum near Peyer's patches in the small intestine by immunofluorescence test. The preparation of histological slices of the different groups under study were as described Perdigón *et al.* (1995). The direct immunofluorescence test was performed using the respective monospecific antibodies (α -chain specific) conjugated with fluorescein isothiocyanate (FITC) (Sigma, St. Louis, MO 61378 USA) or FITC conjugates monoclonal antibodies specific for $CD4^+$ or $CD8^+$ T lymphocytes (Gibco BRD Life Technologies, Neutroquimica SA, Buenos Aires 1125, Argentina). Histological samples of small intestine were incubated with 0.2 ml of different antibodies at 1/100 dilutions for IgA or 1/200 for $CD4^+$ and $CD8^+$ during 30 min at room temperature. Then, they were washed three times with 0.01 M Na phosphate-buffered saline, pH = 7.2. Negative controls were run using the respective unlabelled antibodies (α chain specific or $CD4^+$, $CD8^+$) before incubation with FITC-conjugated antibodies. The results were expressed as the mean of number of positive cells *per* 10 fields (magnification 100x). They represent the mean of three histological slices of each animals (n = 3), for each period of administration and LAB.

Histological samples

Histological slices were also stained with haematoxylin-eosin to identify macrophages, eosinophils, polymorphonuclear (PMN) cells and intraepithelial lymphocytes (IEL). The results were expressed as number of cells on 10 villi and *per* 10 fields (magnification 100 x) for IEL. Values represent the mean of three determination from each animal (n = 3) for each period of feeding and LAB.

Histochemical staining

In order to study the mast cells associated with the intestinal epithelium, the histological samples were stained with Alcian-Bluc-Safranine at pH = 1.5, according Tass (1977) methodology. The number of mast cells were expressed *per* 10 fields (magnification 100x). Three histological slices from each animals (n = 2) period of administration and LAB, were analized.

Statistical Analysis

Data were expressed as the mean (M) of n independent experiments ± standard errors of the mean (SEM). Student's test was used to calculate the statistical significance of the results.

Results

Determination of IgA producing cells on lamina propria of small intestine

A different pattern in the number of IgA^+ cells through the administration time for each strain was observed. Comparatively to the normal control, we observed that *L. rhamnosus* increased the number of IgA^+ cells with the time of administration. *L. acidophilus* and *L. casei* showed an increase for 2 d of feeding, for 5 or 7 d the values decreased being similar to the normal control. *L. delbrueckii* ssp. *bulgaricus* induced an increase on the IgA cells for all the doses, *S. thermophilus* for 2 and 7 d while *Lac. lactis* showed a slight increase for 5 d and *L. plantarum* enhanced these cells only for 2 d of administration then the value decreased being less than the normal control.

These results are shown in Table 1.

TABLE 1.

Effect of lactic acid bacteria on the IgA secreting cells associated to the small intestine

	IgA secreting cells (Number/10 fields)		
	Days of administration		
Strains	**2**	**5**	**7**
L. rhamnosus	78 ± 5	104* ± 5	112* ± 6
L. acidophilus	131* ± 7	93 ± 6	86 ± 5
L. casei	118* ± 7	68 ± 6	87 ± 4
L. bulgaricus	135* ± 7	102 ± 7	146* ± 6
S. thermophilus	112* ± 5	86 ± 6	120* ± 7
Lac. lactis	93 ± 6	102 ± 5	65 ± 6
L. plantarum	144* ± 7	50 ± 5	40 ± 4

Normal control: 85 ± 5

Histological slices were performed after LAB administration as described in the text. IgA secreting cells were determined by direct Immmunofluorescence test. Values are the mean of n mice = 5 ± SD.* $p < 0.05$

TABLE 2.

Determination of CD4$^+$ T cells on lamina propria of small intestine, after LAB administration.

	CD4$^+$ T cells (Number/10 fields)		
	Days of administration		
Strains	**2**	**5**	**7**
L. rhamnosus	66 ± 5	24* ± 4	26* ± 5
L. acidophilus	38* ± 4	62 ± 6	65 ± 7
L. casei	131** ± 5	95** ± 5	49 ± 7
L. bulgaricus	31* ± 4	37 ± 5	40 ± 4
Lac. lactis	42 ± 7	62 ± 7	25* ± 4
S. thermophilus	49 ± 6	39* ± 6	39 ± 7
L. plantarum	82** ± 7	29* ± 4	31* ± 5

Normal control: 54 ± 4

LAB were administered for 2, 5 or 7 consecutive days. CD4$^+$ T cells were determined at the end of each administration period by Immunofluorescence test on histological slices from small intestine. Results are expressed as mean of n mice= 5 ± SD. *Values significant lower than the control with p between < 0.05 and < 0.01. **Significant higher values than the control with p between < 0.01 and < 0.001.

TABLE 3.

Effect of LAB administration on CD8$^+$ T cells number from small intestine

	CD8$^+$ T cells (Number/10 fields)		
	Days of administration		
Strains	**2**	**5**	**7**
L. rhamnosus	28* ± 4	22* ± 4	27* ± 5
L. acidophilus	64 ± 6	40 ± 6	39 ± 5
L. casei	50 ± 5	55± 6	49 ± 5
L. bulgaricus	35* ± 7	41± 6	50 ± 7
S. thermophilus	49 ± 5	31*± 5	38*± 7
Lac. lactis	41 ± 6	54 ± 5	38*± 5
L. plantarum	107** ± 8	38*± 7	33*± 6

Normal control: 58 ± 5

CD8$^+$ T cells were counted on lamina propria of histological slices from small intestine by direct Immunofluorescence test. Results are mean of n mice = 5 ± SD. *Values significant lower than the control with p < 0.01 and < 0.001. **Significant higher values related to the normal control < 0.01.

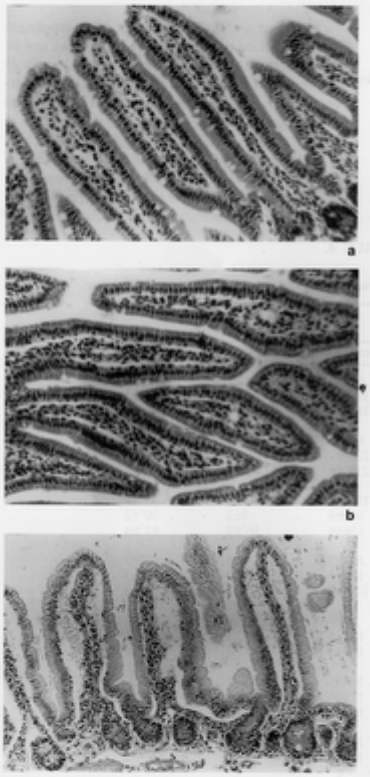

FIGURE 1. Light micrograph of paraffin sections stained with haematoxylin-eosin from small intestine. They show an increase in the number of immune cells associated to the gut (b) or inflammatory response with oedema in the villi (c).
Magnification 40 x.
a) Normal mice; b) Treated with *L. casei* during 2 days; c) Treated with *L. plantarum* during 5 days.

Identification of CD4⁺ and CD8⁺ T cells on lamina propria of small intestine

When we determined CD4⁺ T cells we observed that *L. casei* was able to increase this population for the doses of 2 and 5 d related to the normal control, while *L. plantarum* only did for two days of administration, then the values were lower than the control. *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* disminished these T cells for all the doses assayed. *L. rhamnosus*, *L. acidophilus* and *Lac. lactis* did not show an increase in CD4⁺ T cells and the values were lower or similar than the control (see Table 2).

The values obtained for CD8⁺ T cells were lower than the control values in the cases of *L.delbruekii* ssp. *bulgaricus*, *S. thermophilus*, *L. rhamnosus* and *Lac. lactis*. For *L. casei* there was not modification as regard to the control values. *L. acidophilus* showed a not significance increase for 2 d while *L. plantarum* induced a significant increase for 2 d, then they were lower than the control. These results are expressed in Table 3.

Quantification of inflammatory immune cells on lamina propria of small intestine

When we analyzed the number of macrophages present on lamina propria of small intestine we saw an increase of them in relation to the control values for *L. rhamnosus* and *Lac. lactis* for 5 and 7 d of administration. *L. delbruekii* ssp. *bulgaricus*, *S. thermophilus* did not induce augmentation on the macrophage number in the doses assayed. We observed a slight increase for 7 d of *L. casei* and for 2 d of *L. acidophilus*. In the case of *L. plantarum* the values were lower than the control one for 2 and 5 d. There were not modification in the number of polymorphonuclears (PMN) in relation with the

TABLE 4.

Effect of LAB on the immune cells involved in the inflammatory immune response.

Strains	Days of administration	Cells/10 villi		
		Macrophages	PMN	Eosinophils
L.rhamnosus	2	111±5	9 ±2	19*±2
	5	172*±4	21*±3	19*±2
	7	169*±6	13*±2	14±2
Lac.lactis	2	114±4	5±1	13±2
	5	220*±7	26*±2	30*±2
	7	219*±6	28*±3	33*±2
L.acidophilus	2	191*±7	11±2	33*±3
	5	143±6	8±2	30*±2
	7	156±6	16*±2	61*±4
L.casei	2	133±7	9±2	22*±3
	5	110±5	9±2	19*±3
	7	170*±7	4±1	19*±2
L.bulgaricus	2	91±4	26*±3	20*±2
	5	115±6	16*±2	15±2
	7	100±5	13*±2	3±1
S.thermophilus	2	108±6	22*±2	11±2
	5	119±6	20*±2	4±2
	7	107±6	21*±2	13±2
L.plantarum	2	121±7	23*±3	11±2
	5	106±6	17*±2	7±2
	7	-	-	-

Control Values: Macrophages = 143 ± 6
Polymorphonuclears (PMN) = 6 ± 2
Eosinophils = 10 ± 3

Inflammatory cells were determined on histological slices from small intestine stained with haematoxylin-eosin. Results are mean of n mice = 5 ± SD. *Significant values $p < 0.01$.

control for *L. casei* in all of the dose assayed. *L. acidophilus* increased the number of PMN for 7 d of administration while *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus*, *L. rhamnosus*, *Lac. lactis* and *L. plantarum* showed PMN cells increase. As regard to the eosinophils number they were increased for *L. acidophilus*, *L. casei*, *L. rhamnosus* and *Lac. lactis* for all the period assayed. *L. delbrueckii* ssp. *bulgaricus* showed an increase only for 2 d. We did not observe eosinophils increase with *S. thermophilus* and *L. plantarum* administration. However for 7 d of *L. plantarum* we could not identify the immune cells due to necrosis. These results are expressed in Table 4. The histological alteration observed when the LAB induced an increase in the number of cells involved inflammatory immune response are showed in Fig. 1 a, b and c. The oedema in the villi is remarkable.

Determination of IEL and mast cell associated with the intestinal epithelium

We observed that most of the LAB assayed increase the number of IEL as regard to the control values. The increase was dose dependent. *L. rhamnosus*, *Lac. Lactis* and *L. casei* shown diminished or similar values to the control. *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus*, *L. acidophilus* and *L. plantarum* induced an enhance of those cells depending of the dose assayed. When we determined by Alcian-Blue staining if some of the intraepithelial lymphoid cells were mast cells, we observed that *L. rhamnosus* and *L. acidophilus* increased these cells only for 7 days. *Lac. lactis*, *S. thermophilus*, *L. casei* and *L. plantarum* did not increase the mast cells. *L. bulgaricus* showed a significant enhance for 5 and 7 days.

These results are shown in Table 5.

TABLE 5.

Effect of LAB on the number of IEL and Mast cells associated to intestinal epithelium

Strains	Days of administration	Cells/10 fields	
		IEL	Mast cells
L.rhamnosus	2	30.5±3.4	20.22±2.3
	5	53±4.1	19.16±2.8
	7	33.5±2.8	51.17*±2.1
Lac.lactis	2	48.66±3.8	20.83±3.2
	5	58.16*±4.2	30±5
	7	25±4	20.83±3.2
L.acidophilus	2	75.33**±3.2	10.17±3
	5	67.33*±4.3	21.33±2
	7	63.33*±4.1	70.16**±3.1
L.casei	2	38±5.2	21.33±1.7
	5	32.33±6.1	33.1±3.1
	7	37.65±3.1	13±2.4
L.bulgaricus	2	43.33±4.1	12.67±2.1
	5	198.67**±6.2	55.66*±4.1
	7	103.33**±5.1	39.77*±2.3
S.thermophilus	2	60.66*±5.1	18±1.8
	5	38.33±3.3	16.67±5.71
	7	68.33*±6.2	24.16±1.8
L.plantarum	2	61.33*±3.4	19±1.8
	5	131.67**±2.1	17.33±4.3
	7	56.19±3.2	17.1±3.2

Control values: Intraepithelial lymphocytes (IEL) = 45.66 ± 4.1
Mast cells = 20 ± 2

Results are means of n mice = 5 ± SD. Significant values * $p < 0.05$, ** $p < 0.01$

Discussion

The intestinal immune system consists of a number of compartments with different functions contributing to a mucosal immune response. They are: Peyer's patches (PP), gut lamina propria and intra-epithelial lymphocytes (Weinstein and Cebra, 1991; Mc Ghee *et al.*, 1985). Peyer's patches are the inductive site for the generation of IgA committed, preplasmablast and IgA memory cells (Cebra and Shroff, 1994). In this site the antigens are transported through specialized cells called M cells which put in contact the antigen with the antigen presenting cells (APC) to be processed and presented to $CD4^+$ T cells (Walker and Sanderson, 1995). Antigen specific IgA committed B cells and plasmablasts emigrate to the mesenteric lymph nodes, where further expression and maturation of these cells may occur. T cells are also able to migrate (Cerf-Bensussan, 1995). The intestinal lamina propria (LP) receives pre plasma cells from both PP and mesenteric lymph nodes (MLN) (Weiner, 1997), it also receives $CD4^+$ and $CD8^+$ T cells antigen specific or polyclonally induced and non specific NK cells. Antigen specific $CD8^+$ T cells may be generated in PP and emigrate to the intra-epithelial space. In LP are also the immune cells involved in the inflammatory immune response. However, intestinal immunization can induce a profound suppression of the local and also the systemic immune response as just has been reported (Weiner, 1997; Tomasi, 1980). This phenomenon is called oral tolerance and appears to be mediated by cytokines released from immune cells.

In the present paper we studied how the oral administration of LAB interacting with the gut can influence this complexe network of signals between epithelial and immune cells. It was also studied whether or not these microorganisms are able to activate the mucosal immune system and what kind of immune response is induced.

When we analyzed the behaviour of the different LAB assayed on the immune cells associated to mucosa, we saw that each strain showed a different pattern of immune cells activation, especially in the case of IgA producing cells, where this increase, in the most of the LAB studied, was not correlated with an increase in $CD4^+$ T lymphocytes (see Table 1 and 2). It is known the role that plays $CD4^+$ T cell in increasing of IgA^+ B cells favouring the switch IgM to IgA (Weinstein and Cebra, 1991). It was also demonstrated that macrophages and intestinal epithelial cells by antigen stimulation release interleukin 6 (IL6), which can induce IgA^+ cell expression and selective synthesis of this immunoglobulin (Fujihashi *et al.*, 1991). We believe that those LAB that were not able to increase $CD4^+$ T cells, the IgA^+ B cells were enhanced as a consequence of other mechanisms, for example, through IL6 release or by clonal expansion of IgA^+ B cells present in lamina propria. As regard $CD8^+$, with exception of *L. plantarum* cytotoxic T cells were not stimulated. The values were disminished in almost all the LAB assayed (Table 3). This fact, is possitive for the host in order to avoid the cytotoxic immune response. In the case of *L. plantarum*, the necrosis observed for 7 d may be due to an increase in the cytotoxic mechanisms.

When we determined the immune cells involved in the inflammatory immune response we observed an increase in the number of macrophages, neutrophils and eosinophils for the most of the LAB under study (Table 4). These observations would mean an enhance in the inflammatory immune response, which can modify the histological structure of the small intestine (Fig. 1). The increase in the inflammatory cells can also affect the intestinal permeability through the release of substances biologically active as cytokines, produced by cells present in the lamina propria such as PMN, eosinophils or Th1 lymphocytes (Stallmach *et al.*, 1988). Macrophages augmentation would be a possitive fact considering the multiple functions of this cell in the host defense such as phagocytosis of opportunistic, non-pathogenic and pathogenic microorganisms that cross the intestinal barrier to the lamina propria of the gut, where they are phagocyted and killed after phagocytosis (Wells *et al.*, 1988a). We believe that the induction of an inflammatory immune response could favour the uptake of bacteria (translocation of normal microflora) or other antigens present in the intestinal lumen (Wells *et al.*, 1988b). The correlation between inflammatory response and bacterial translocation has been demonstrated in acute inflammation caused by *Shigella* infection (Zychlinsky *et al.*, 1996).

However, it is well demonstrated the role of the mucus layer, which is produced mainly by globet cells after inflammatory stimulus. Mucus is not digested because of its resistance to various enzymes and can protect epithelium against the adherence of pathogens. Mucus secretion can be triggered by direct stimulation of immune complexes and chemical agents and by indirect stimulation by mediator release, such as histamine and lymphokines (Snyder and Walker, 1987).

The effect of various cytokines and growth factors released by immune cells associated to intestinal mucosal, depending of the dose may serve as important modulators of epithelial cell function. Dignass and

Podalsky (1993) demonstrated that some cytokines such us Transforming Growth Factor (TGFβ), cytokines such as IL1β, IFNγ, TNFα and some prostaglandins (PGF) enhanced epithelial cell restitution and they play an important role in sustaining normal mucosal.

The IEL which are $CD8^+$ cells play an important role as protective barrier against infections specially those with T cell receptor (TCR) γ/δ chains (De Libero, 1997). Although we did not determine TCR of IEL some LAB, even when they increased the number of these immune cells associated with the intestine, not all were IEL, we demonstrated the presence of mast cell associated to the gut epithelium. The biological significance of these cells in our experimental model must be determined.

According our results, to assure that certain LAB are able to exert a beneficial effect on the gut, we think that would be important to determine, as a bearing parameter, if the strain assayed is or not able to induce a high inflammatory stimulus to avoid side effects. The inflammatory stimulus could be diminished by decreasing of the daily dose of LAB to be administered, improving the mucosal immunity. As was explained before, a low inflammatory immune response induces beneficial effect in the host. It is also known that an increase in the non specific immune response mediated by PMN would be important by the participation of these cells populations as the first line in the host defense against enteric infections as has been just described (Kagnoff and Eckmann, 1997).

To the light of our results, although the LAB assayed (*L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. plantarum*, *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus*, *Lac. lactis*) increased the number of IgA immune cells associated to gut, depending of the dose assayed -some of them could induce an enhance in the inflammatory response. The different mucosal immune responses observed, might be due to the different way by which the LAB interact with the small intestine. This last speculation is based in previous reports (Walker and Sanderson, 1995; Weiner, 1997) related to the different ways by which an antigen can interact with the immune cells associated to the gut.

Futher studies for *in vivo* cytokines determination are needed to determine if the $CD4^+$ T cells detected are Th1 or Th2 type, or if the LAB stimulation enhance the release of proinflammatory cytokines such as TNF α or IFN γ, thus increasing the inflammatory response.

We determined the importance in the selection of LAB and the dose to be administered exerting a beneficial effect on the intestine. We also demonstrated that the immunostimulatory capacity of the LAB can not be generalyzed to genus or specie. This property would be restricted to the strain assayed, and not all of the LAB could be use to enhance the intestinal immuntiy.

Acknowledgements

We thank Lic. E. Bru de Labanda for the statistical studies. This work was supported by Grant from Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT) 26/D127, PIP 5011 from CONICET, and PICT 97/05-02312.

References

AGERBACK M, GERDES U, RICLELSEN B (1995). Hypocholesterolemic effect of a new fermented milk product in healthly middle aged men. Eur J Clinical Nutr 49: 346-352.

CEBRA JJ, SHROFF K (1994). Peyer's patches as inductive sites for IgA commitment. In: Handbook of Mucosal Immunology. P.L. Ogra, M.E. Lamm, W. Strober, J.R. Mc Ghee, J. Bienenstock, Eds. Academic Press. San Diego, USA, pp. 151-158.

CERF-BENSUSSAN N (1995). Mucosal intestinal T lymphocytes. In: Mucosal Immunity and the Gut Epithelium: Interactions in Health and Disease. S. Auricchio, A. Ferguson, R. Troncone, Eds. Dynamic Nutrition Research Basel, Karger, pp. 40-49.

DE LIBERO G (1997). Sentinel function of broadly reactive human g d T cells. Immunol Today 18: 22-26.

DE MAN JC, ROGOSA M, SHARPE ME (1960). A medium for the cultivation of lactobacilli. J Applied Bacteriol 23: 130-155.

DIGNASS A, PODALSKY D (1993). Cytokine modulation of intestinal epithelial cell restitution control role of transforming growth factor B. Gastroenterology 105: 1323-1332.

FUJIHASHI K, MC GHEE J, LUE C, BEAGLEY K, TAGA T, KISHIMOTO T, MESTECKY J, KIYONO H (1991). Human appendix B cells naturally express receptors for and respond to interleukin 6 with selective IgA_1 and IgA_2 synthesis. J Clin Invest 88: 248-252.

FULLER R (1992). *History and development of probiotics.* In: Probiotics. R. Fuller, Ed. London. Chapman and Hall, pp. 2-8.

HAMILTON-MILLER JMT (1996). Probiotics panacea or nostrum? BNF Nutr -Bull 21: 199-203.

ISOLAURI E, JUNTUNEN M, RAUTANEN T, SILLANAUKEE P, KOIVULA T (1991). A human *Lactobacillus* strain (Lactobacillus GG) promotes recovery from acute diarrohea in children. Pediatrics. 88: 90-97.

KAGNOFF M, ECKMANN L (1997). Epithelial cells: Chemokine production in response to microbial infection. Mucosal Immunol. Up Date 5: 41-44.

KLASEN HLBM, VAN DER HEIJDEN PJ, STOK W, POELMA FGJ, KOOPMAN JP, VAN DE BRINK ME, BAKKER MH, ELING

WMV, BEYNEN AC (1993). Apathogenic intestinal segmented filamentous bacteria stimulate the mucosal immune system. Infect Immun 61: 303-306.

MATZUSAKI T, YOKOKURA T, AZUMA I (1985). Antitumour activity of *Lactobacillus casei* on Lewis lung carcinoma and line 10 hepatoma in singeneic mice and guinea pigs. Cancer Immunol Immunoth 20: 18-22.

MC GHEE JR, MICHALEK SM, KIYONO H, WILLIAMSON SI, BROWN TA, MESTECKY J (1985). *Mucosal immunoregulation: IgA inductive sites, isotype-specific helper T cells, gut LPS influence and subclass distribution of IgA antibodies*. In: the Pathogenesis of Bacterial Infectious. G.G. Jookson and H. Thomas, Eds. Springer-Verlag Berlin, Heidelberg, New York. Tokyo, pp. 85-88.

MOREAU MC, DUCLUZEAUD R, GUY GRAND D, MULLER MC (1978). Increase in the population of duodenal immunoglobulin A plasmocytes in axenic mice associated with different living or dead bacterial strains of intestinal origin. Infect Immun 21: 532-539.

PERDIGÓN G, ALVAREZ S, GOBBATO N, VALVERDE DE BUDEGUER M, DE RUIZ HOLGADO A P (1995). Comparative effect of the adjuvant capacity of *L. casei* and LPS on the intestinal secretory antibody response and resistance to *Salmonella* infection in mice. J Food Agricultural Immunol 7: 283-294.

PERDIGÓN G, ALVAREZ S, DE RUIZ HOLGADO AP (1991). Oral immunoadjuvant activity of *L. casei* influence of the dose administered on the secretory immune response and protective capacity in intestinal infections. J Dairy Res 58: 485-496.

PERDIGÓN G, NADER DE MACIAS ME, ALVAREZ S, OLIVER G, DE RUIZ HOLGADO AP (1986). Effect of perorally administered Lactobacilli on macrophage activation in mice. Infect Immun 53: 404-410.

PERDIGÓN G, NADER DE MACÍAS ME, ALVAREZ S, OLIVER G, DE RUIZ HOLGADO AP (1988). Systemic augmentation of immune response in mice by feeding fermented milk with *L. casei* and *L. acidophilus*. Immunol 63: 17-23.

PERDIGÓN G, VALDEZ J, RACHID M (1998). Antitumour activity of yogurt: study of possible immune mechanisms. J Dairy Res 65: 129-138.

RAIBAUD P, CAULET M, GALPEN JV, MOCQUOT G (1961). Studies on the bacterial flora of the alimentary tract of pigs II. Streptococci: selective enumeration and differentiation of the dominant group. Appl Bacteriol 24: 285-291.

SAAVEDRA JM, BAUMAN NA, OUNG I, PERMAN JA, YOLKEN RH (1994). Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 344: 1046-1049.

SANDERS ME (1993). Effect of consumption of lactic cultures on human health. Adv Food Nutr Res 17: 67-130.

SCHIFFRIN EJ, ROCHAT F, LINK-AMSTER H, AESCHLIMANN J, DONNET-HUGHES A (1995). Immunomodulation of human blood cells following the ingestion of Lactic acid bacteria. J Dairy Sci 78: 491-497.

SNYDER JD, WALKER WA (1987). Structure and function of intestinal mucin: development aspect. Int Arch Allergy Appl Immunol 92: 351-356.

STALLMACH A, STROBER W, MAC DONALD T, LOCHS H, ZEITS M (1998). Induction and modulation of gastrointestinal inflammation. Immun Today 19: 438-441.

TASS J (1997). The alcian blue and combined alcian blue.safranine staining of glycosamino glycans studied in a model system and in mast cells. Histochemical Journal 9: 205-210.

TOMASI TB (1980). Oral tolerance. Transplantation. 29: 353-356.

WALKER WA, SANDERSON IR (1995). *The enterocyte and antigen transport*. In: Mucosal Immunity and the Gut Epithelium: Interactions in Health and Disease. S. Auricchio, A. Ferguson A. and R. Troncone, Eds. Dynamic Nutrition Research. Basel, Karger, pp. 18-31.

WEINER H (1997). Oral tolerance: immune mechanisms and treatment of autoimmune diseases. Immun Today 18: 335-343.

WEINSTEIN PD, CEBRA JJ (1991). The preference for switching to IgA expression by Peyer's patch germinal center B cells is likely due to the intrinsic influence of their microenvironment. J Immunol 147: 4126-4135.

WELLS CL, MADDAUS MA, ERLANDSEN S, SIMMONS RL (1988a). Evidence for phagocytic transport of intestinal particles in dog and rats. Infect Immun 56: 278-282.

WELLS CL, MADDAUS MA, SIMMONS RL (1988 b). Proposed mechanisms for the translocation of intestinal bacteria. Rev Infect Dis 10: 958-979.

ZYCHLINSKY A, THIRUMALAI K, ARONDEL J, CANTEY R, ALIPRANTIS A, SANSONE P (1996). *In vivo* apoptosis in Shigella flexneri infections. Infect Immun 64: 5357-5365.