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IMMUNOMODULATORY AND ANTI-INFLAMMATORY ACTIVITY INDUCED BY ORAL ADMINISTRATION OF A PROBIOTIC STRAIN OF *LACTOBACILLUS CASEI*

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The aim of this work was to study the effect of the long-term cyclic administration of the probiotic strain of *Lactobacillus* (*L.*) *casei* CRL 431 as a mucosal immunomodulator of the immune cells associated with the lamina propria of the small intestine, bronchus and other immune cells not associated with mucosal tissues, such as peritoneal macrophages. BALB/c mice were orally administered with a suspension of *Lc* 10^9 cfu/day/animal in non-fat milk (NFM) 10% for two consecutive days, the optimal dose selected in previous studies to reach protective immunity. This administration was repeated cyclically every 5 days for 98 days. Mice in the control group received only NFM 10%. Samples were taken after two days of *L. casei* administration and every 14 days until day 98. The small intestine and lungs were removed for histological slices preparation. Haematoxilin-eosin stains were made for histological studies of the small intestine. The number of IgA producing cells in the lamina propria of the small intestine and in bronchus was determined by immunofluorescence assays. Regulatory (IL-4, IL-10) and proinflammatory (TNF-α, INF-γ) cytokines were measured in the gut. Peritoneal macrophages were collected during the same periods for phagocytosis assays. We determined an increase in the number of IgA^+ cells in the lamina propria of the small intestine in all the periods assayed and in BALT only until day 28. The cytokines studied (IL-10, IL-4, TNF-α and INF-γ) increased in most of the periods assayed, the effect being more remarkable for the anti-inflammatory cytokines such as IL-4 and IL-10. INF-γ was also increased but no modifications in the histological studies of the small intestine were observed, suggesting other roles for this cytokine The phagocytic activity of PM increased for most of the periods assayed. We demonstrated that long-term cyclic oral *L. casei* administration favors mucosal immunity and modulates the immune response to maintain the homeostasis at the mucosal level.

The immunological properties of probiotic bacteria have been extensively studied. Certain non-pathogenic lactic acid bacteria (LAB) such as *Lactobacillus* (*L.*) *casei, L. rhamnosus and L. plantarum* enhance both the systemic and the mucosal immunity (1). Some LAB strains administered by the oral route have been proved to efficiently stimulate the phagocytic activity of PMN from peripheral blood cells (2-3). Studies of the intestinal mucosal immune system have shown that not all LAB can act as immune potentiators, since this property would be related to the strain assayed rather than to the bacterial genus or species (4).

In previous studies we demonstrated that the oral administration of *L. casei* CRL 431 induced anti-tumor immune mechanisms that inhibited

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the progression of a subcutaneously implanted fibrosarcoma, the effect of the microorganism being intimately related to its administration dose (2×10^9 cfu ml^{-1}) (5-6). The anti-infective effect of the induction of high levels of the specific anti-pathogen secretory IgA (S-IgA) against *Salmonella typhimurium* and *Escherichia coli* infection was also demonstrated (7). We noticed the importance of the administration of 2×10^9 cells of *L. casei* in order to prevent infection against enteropathogens (8-9). We demonstrated for short periods of time (2, 5 or 7 days) that orally administered live LAB can modulate the gut mucosal immune response according to the elicited cytokine profiles and that the immune response obtained is closely related to the dose administered (10), as demonstrated by other authors (11), also for short periods of time.

In most papers dealing with the use of LAB as an immunopotentiator of the gut- associated lymphoid tissue (GALT), the appropriate dose for good activation of the mucosal system is emphasized to prevent oral tolerance mechanisms (12). However, no scientific evidence has been provided as to whether or not these microorganisms are able to immunomodulate the immune response beyond the optimal dose administered cyclically. Evidence is also lacking as to whether the upregulation of the immune system reached with the optimal dose is maintained throughout long-term cyclic stimulation or whether down regulation occurs.

In a previous paper we determined that *L. casei* interacts with the GALT and makes contact with the immune cells associated with Peyer's patches and with the lamina propria of the intestinal mucosa (13). We also demonstrated that *L. casei* interacts with the surface of the epithelial cells and that only small antigenic particle from the cell wall or cytoplasmic substances, but not the whole bacterium, were capable of penetrating the epithelial barrier and making contact with the immune cells associated with the intestine. We confirmed that bacterial viability was also an important condition for a better stimulation of the GALT (14). These previous studies, and the observation that lower doses of *L. casei* were more effective than higher doses to reach protective immunity by the oral route, led us to analyze the immunomodulatory capacity of *L. casei* cyclically administered every 5 days for long periods of time after previous stimulation with the optimal accumulative dose (2×10^9 cfu ml^{-1}). We studied the influence of such administration on the immune cells associated with the lamina propria of the small intestine and whether or not this cyclic intestinal stimulation for long periods of time had an effect on the IgA^+ cells in distant mucosal sites such as bronchus or on other immune cells not associated with mucosal tissues such as peritoneal macrophages, using a murine experimental model.

MATERIALS AND METHODS

Animals

Six-week-old BALB/c mice weighing 25-28 g were obtained from the random-bred colony kept in our department at the Institute of Microbiology (Facultad de Bioquímica, Química y Farmacia – UNT). Each experimental group consisted of a total of 30 animals. Three to five mice per assay and period of time were used. Each assay was performed by duplicate or triplicate. All animal protocols were approved by the Animal Protection Committee of CERELA and Universidad Nacional de Tucumán. All experiments comply with the current laws of Argentina.

Microorganisms

The *Lactobacillus* strain used for experiments was *L. casei* CRL 431, from the CERELA culture collection. This microorganism was selected by its immunological properties, as demonstrated in previous papers (7-10). The microorganism was cultured for 18 h at 37°C in MRS broth (aerobic conditions). After that, *L. casei* was harvested by centrifugation at 5,000 *g* for 10 min and washed three times with sterile saline solution (0.85% NaCl).

L. casei administration procedure

The mice were separated into two experimental groups. The animals in the control group were given 3 ml non-fat milk (NFM) at 10% in their drinking water and received a conventional balanced diet *ad libitum*. The test group animals were administered daily with 3 ml of *L. casei* suspended in 5 ml sterile NFM 10% at a concentration of 10^9 cfu ml^{-1} for two consecutive days (optimal dose, 2×10^9 cfu ml^{-1}). This administration was repeated cyclically every 5 days until day 98 (14 weeks). Continuous *L. casei* administration was not used since previous findings in our laboratory proved that doses of over two days were not protective against enteropathogens infection or antitumour activity (5-7). The culture suspension was administered at 20% v/v in the drinking water, the intake being 3 ml/day/mouse. The number of viable bacteria was controlled daily by standard plate count technique. The mice for the different assays were housed in individual cages. All the groups received a conventional balanced diet *ad libitum*.

Histological samples

The mice from both groups were killed by cervical dislocation. Samples were taken after two days of *L. casei* administration, and then on day 14 and every 14 days until day 98. The small intestine and lungs were removed. The intestinal content was eliminated by washing with saline solution (0.85% NaCl). Tissues were prepared for histological evaluation using the routine methods for paraffin inclusion according to Sainte Marie's Technique (15). Four μm serial paraffin sections were cut. Slices from the gut were stained with haematoxylin-eosin for light microscopy examination to determine possible histological modifications in the gut. Histological slices from the small intestine and lung were used for immunofluorescence assays.

Immunofluorescence assay for IgA-producing cells determination

The number of B cells secreting IgA was determined in the lamina propria of the ileum region near the Peyer's patches and in the bronchus-associated lymphoid tissue (BALT) of the lungs in the histological slices of the samples from both control and test groups for each period of *L. casei* administration. IgA^+ secreting cells were measured using α-chain monospecific antibody conjugated with fluorescein isothiocyanate (FITC) (Sigma, St. Louis, MO, USA). Samples were deparaffined and incubated with the appropriate antibody dilution (1:100) in 0.01 mol l^{-1} Na phosphate buffered saline (PBS) solution, pH 7.2, for 30 min at 37°C. They were then washed 3 times with PBS solution. The number of fluorescent cells was counted in thirty fields of vision as seen at 1000X magnification using a fluorescent light microscope. The results were expressed as number of positive cells in ten fields. They represent the mean of three histological slices from each animal (n=3) for each feeding period.

Indirect immunofluorescence detection of cytokine-producing cells

Cytokines were determined in the small intestine of both test and control animals during the same periods as for IgA^+ cell determination. TNF-α, INF-γ, IL-4 and IL-10 producing cells were determined in the histological slices by indirect immunofluorescence test, using specific rabbit anti-mouse cytokine polyclonal antibody diluted in saponin-Hank`s balanced saline solution (HBSS) and a goat anti-rabbit antibody conjugated with FITC (Jackson Immuno Research Lab Inc., West Grove, USA). The deparaffined histological samples were incubated with the appropriate 1st antibody dilution (1:100 for each cytokine assayed) in 0.01 mol l^{-1} PBS solution, pH 7.2, for 60 min at 37 °C. They were then washed three times with PBS solution and incubated with the 2nd fluorescent antibody dilution (1:100) for 60 min at 37°C. The number of fluorescent cells for each cytokine was counted in the lamina propria of the small intestine of 3 samples from each group and period of time assayed in 30 fields of vision at 1000X magnification. The results were expressed as the mean of the number of cells in 10 fields.

Ex vivo phagocytosis assay

Peritoneal macrophages were aseptically collected from the test and control groups of mice at the same periods as for IgA and cytokines-secreting cells determination. They were washed twice with PBS plus bovine seroalbumin (BSA) at 1% and adjusted to a concentration of 10^6 cells ml^{-1}. Phagocytosis was performed using a heat-killed *Candida albicans* suspension (100°C, 15 min) at a concentration of 10^7 cell ml^{-1}. Mixtures of opsonized *Candida* with the mouse autologous serum (10%) were added to 0.2 ml of macrophage suspension. The mixture was incubated for 30 min at 37°C. The percentage of phagocytosis was expressed as the percentage of phagocyting macrophages in 200-cell count using an optical microscope.

Statistical analysis

Data were expressed as the mean of n=3 independent experiments ± standard errors (SEM). Student's T test was used to calculate the statistical significance of the results, comparing the group of mice fed with *L. casei* with the control groups.

RESULTS

Histological studies of the small intestine

L. casei cyclically administered for long periods of time did not induce secondary effects such as an inflammatory immune response at the gut level. As shown in Fig. 1a and 1b, a slight cellular infiltration in the lamina propria of the small intestine with a conserved histological structure of the villi was seen in the animals with cyclic administration of *L. casei* in relation to the NFM control group during most of the periods assayed. The increase in cellularity was observed until day 42, after which the infiltrative cells diminished.

Effect of long-term cyclic oral administration of L. casei on the IgA^+ cells associated with the lamina propria of the small intestine

When we analyzed the effect of the long-term cyclic administration of the strain assayed, we observed that there was a significant increase in the number of IgA-secreting cells in respect to the control group fed with NFM (10%). Values were significantly higher ($P<0.001$) in mice fed cyclically with *L. casei* with respect to NFM control values.

The increase was maintained at all periods assayed up to day 98, except for day 28, where the values were not significant either with regard to the NFM control group or with the untreated control group. These results are shown in Fig. 2.

Determination of regulatory cytokines (IL-4 and IL-10)

With regard to the regulatory cytokines IL-4 and IL-10 that maintain intestinal homeostasis, we observed that IL-4 in the test group showed a significant difference ($P<0.001$) in respect to the control group at all the periods assayed. The number of IL-4 positive cells showed a greater increase on days 14, 28 and 42. IL-10 was also significantly increased in the groups given *L. casei* with respect to both the NFM control and the untreated control group from the 2nd to the 28th day after treatment. Then values decreased until the end of the experiment, being similar in both controls. Table I shows these results.

Determination of cells producing proinflammatory cytokines TNF-α and IFN-γ at the gut level

Two proinflammatory cytokines, TNF-α and INF-γ were selected to determine whether the cyclic administration of *L. casei* for long periods of time induced high levels in the production of these cytokines, which are responsible for a possible inflammatory response. We observed that TNF-α was significantly increased ($P<0.001$) in the group which received *L. casei* for all the periods assayed. Values reached twice the NFM control group values, this increase being more remarkable on day 28. The levels of IFN-γ in the group fed with *L. casei* were also significantly enhanced ($P<0.001$) from day 2 until day 98 in comparison with both control groups. These results are expressed in Table II.

Effect of L. casei on IgA+ secreting cells in mucosal distant sites such as BALT

The number of IgA secreting cells associated with bronchus was analyzed in order to determine whether or not intestinal stimulation favored the migration of the gut IgA^+ B cells to bronchus. We found that *L. casei* increased the number of IgA+ cells in the bronchus from day 2 to day 28. The effect was highly significant until the 28th day with regard to the NFM control group. After that, and up to day 56, these levels decreased, reaching values similar to both control groups (NFM and unfed control) (Fig. 3).

Effect of long-term of L. casei administration on the phagocytic capacity of peritoneal macrophages

The activity of peritoneal macrophages was studied to determine the activation of cells distant from the intestine that could be stimulated by the cytokines released by the immune cells associated with the gut. We observed that the cyclic oral administration of *L. casei* for long periods of time increased the phagocytic activity of the peritoneal macrophages throughout the 98 days of the experiment. This activity was highly significant from day 2 onwards, with the exception of days 56 and 84, in comparison with the two controls (NFM and unfed control). These results are expressed in Table III.

DISCUSSION

Homeostasis in the immune system of the gastrointestinal tract is sensitively balanced by the immune cells associated with the intestinal mucosa, which are responsible for oral tolerance or for the development of chronic inflammation (16-17). The epithelial layers provide an effective barrier against luminal antigens. The intestinal flora also plays a key role in maintaining the activation of the immune cells associated with the intestine (18). Recently it has been shown that the dendritic cells (DCs) in the intestine are activated by luminal bacterial antigens. Upon activation, they undergo maturation process and migrate to the lymph nodes, where they activate T cells (19). In the Peyer's patches, DCs take up the antigens from the lumen transported by M cells. The dendritic cells of the *lamina propria* of the small intestine express tight junction proteins to prevent the destruction of the epithelial barrier and to be able to take up the antigens (20).

In spite of this knowledge there are very few immunological studies of probiotic bacteria and the way in which these microorganisms influence the mucosal and systemic immune response in a complex environment such as the intestinal ecosystem. The immunological properties of probiotic bacteria have been well demonstrated (2-3, 5-6, 11-13). In this context, and considering that oral tolerance is the mechanism that maintains intestinal homeostasis, many questions arise in relation to the constant

Table I. *Effect of L. casei administration on regulatory cytokines positive cells.*

Veriods assayed	Cytokines			
	IL-4		IL-10	
	L. casei	NFM	*L. casei*	NFM
2 days	90.16 ± 5*	32 ± 7	41.16 ± 2*	30 ± 7
14 days	186 ± 9*	35 ± 5	78.75 ± 4*	28 ± 5
28 days	222.66 ± 19*	35 ± 3	76.04 ± 4*	31 ± 3
42 days	164.75 ± 10*	34 ± 10	32.58 ± 3	33± 10
56 days	97.25 ± 7*	37 ± 8	32.91 ± 3	34 ± 8
70 days	120.66 ± 7*	36 ± 6	34.5 ± 4	30 ± 6
84 days	132.08 ± 10*	31 ± 7	31.87 ± 3	35 ± 7
98 days	127.8 ± 4*	34 ± 9	31.5 ± 2	34 ± 9
Unfed Control	34 ± 2		31 ± 4	

*IL-4 and IL-10 positive cells in the lamina propria of the small intestine from animals with L. casei or NFM administration were determined using indirect immunofluorescence. Values are expressed as mean ± SD of number of positive cells (fluorescent cells) counted in 10 fields of vision at 1000X of magnification (N° of cells/10 fields). * = significant differences of mice fed with L.casei compared with the NFM control (P < 0.001).*

stimulation induced by the probiotic bacteria present in some foods. In this paper we analyzed the effect of long-term (98 days) cyclic oral administration of the probiotic strain *L. casei* CRL 431 at the optimal dose previously determined.

Cyclic administration is also supported by our previous works using this strain (10-14). Long-term administration of viable *L. casei* is justified by the fact that this probiotic strain is able to survive through the intestinal passage interacts with the immune cells, but not to colonize the intestinal tract. We determined that *L. casei* cyclically administered for 98 days did not induce side effects such as an inflammatory immune response even under constant antigenic stimulation. We noticed only a slight increase in the cellularity in the villi and lamina propria of the small intestine (Fig. 1a and 1b).

When we analyzed in the lamina propria of the small intestine the population of IgA producing cells as one of the more important markers of mucosal immunity, we observed that the number of IgA+ cells in the lamina propria (Fig. 2) was increased and remained so until day 98. This fact could mean that long-term cyclic administration of *L. casei* may be necessary to maintain the number of IgA+ cells, which are responsible for the surveillance mechanism in the gut due to their important function at the mucosal level, as demonstrated by Lamm et al. (21).

The activation of the infiltrative immune cells associated to the lamina propria of the small intestine, measured by cytokines positive cells, was also evaluated. We determined how the cytokines' profiles vary after cyclic probiotic administration in order to maintain intestinal homeostasis. For this

Table II. *Effect of L. casei on proinflammatory cytokines positive cells.*

Periods assayed	Cytokines			
	IFN γ		IFN α	
	L. casei	NFM	*L. casei*	NFM
2 days	99.41 ± 11*	50 ± 8	50 ± 6*	23± 3
14 days	90 ± 3*	48 ± 9	58 ± 15*	28 ± 9
28 days	124 ± 13*	55 ± 12	75 ± 23*	24 ± 5
42 days	134.4 ± 14*	56 ± 16	58 ± 7*	26 ± 2
56 days	115.75 ± 7*	54 ± 9	55 ± 18	27 ± 15
70 days	96.5 ± 9*	50 ± 8	49 ± 7*	26 ± 2
84 days	115.66 ±18*	55 ± 9	44 ± 4*	20 ± 5
98 days	96.1 ± 12*	57 ± 5	45 ± 2*	25 ± 3
Unfed Control	51 ± 9		24 ± 4	

INFγ and TNFα regulatory cytokines positive cells in the lamina propria of the small intestine from animals with or without treatment with L. casei were determined using indirect immunofluosrecence. Values are expressed as mean ± SD of number of positive cells (fluorescent cells) counted in 10 fields of vision at 1000X magnification (cells/10 fields). * = *significant differences of mice fed with L. casei compared with the NFM control (P < 0.001).*

reason we selected for analysis two proinflammatory cytokines, TNF-α and INF-γ, and two regulatory cytokines, IL-4 and IL-10 (Tables I and II). We demonstrated that the cells positive for both regulatory cytokines reached a maximum and then, even when their values decreased, they were still significantly increased in respect to the control values, especially for IL-4, perhaps as a way of maintaining intestinal homeostasis against the constant antigenic stimulation by regulation of the Th-1 response. With regard to the proinflammatory cytokines IFN-γ and TNF-α, they were also increased. These cytokines were only partially regulated by IL-10. In the case of INF-γ, the effect was more important than for TNF-α, whose values diminished when those for IL-10 increased. However, TNF-α (+) cell numbers remained high in respect to those of the NFM control. We believe that this cytokine could be responsible for the slight increase in cellularity observed in the lamina propria of the small intestine.

The numbers of INF-γ cells were increased throughout the experiment. We think that the levels found for INF-γ are also responsible for the control of the proliferation of the immune cells in the lamina propria. This speculation is based on the regulatory activity found for INF-γ. It has been demonstrated that Indoleamine dioxygenase (IDO) is an IFN-γ inducible gene and tryptophan-catabolyzing enzyme that inhibits antigen-specific T-cell proliferation (22). The high levels of INF-γ induced by *L. casei* administration would also be important to activate macrophages distant from the gut such as peritoneal macrophages, as shown in the phagocytosis assays (Table III).

Table III. *Effect of long-term cyclic L. casei administration on the phagocytic activity of peritoneal macrophages.*

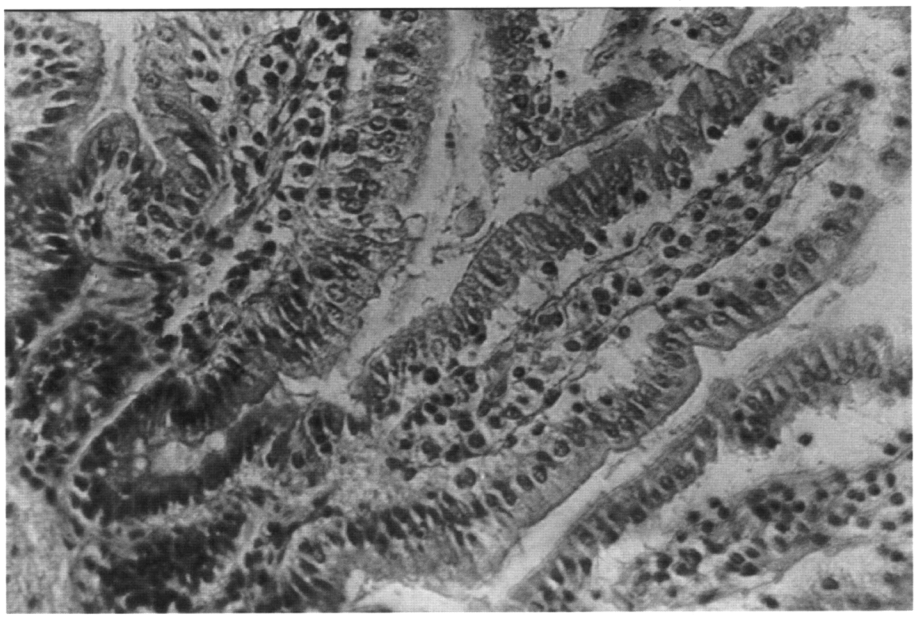
Periods assayed	*L. casei*	NFM
2 days	45± 4*	20± 3
14 days	40± 5*	22± 2
28 days	40± 3*	20± 3
42 days	39± 2*	25± 1
56 days	33± 7	25± 6
70 days	43± 2*	20± 2
84 days	30± 6	20± 5
98 days	35± 3*	20± 2
Unfed Control	22 ± 2	

*Peritoneal macrophages were isolated from test and control groups. The activity of these cells was determined by phagocytosis assay of dead Candida albicans. The values are expressed as mean for n=5 ± SD of percentage of phagocytosis expressed as the percentage of phagocyting macrophages in 200 cells counted. * = significant differences of mice fed with L. casei compared with NFM control (P < 0.001).*

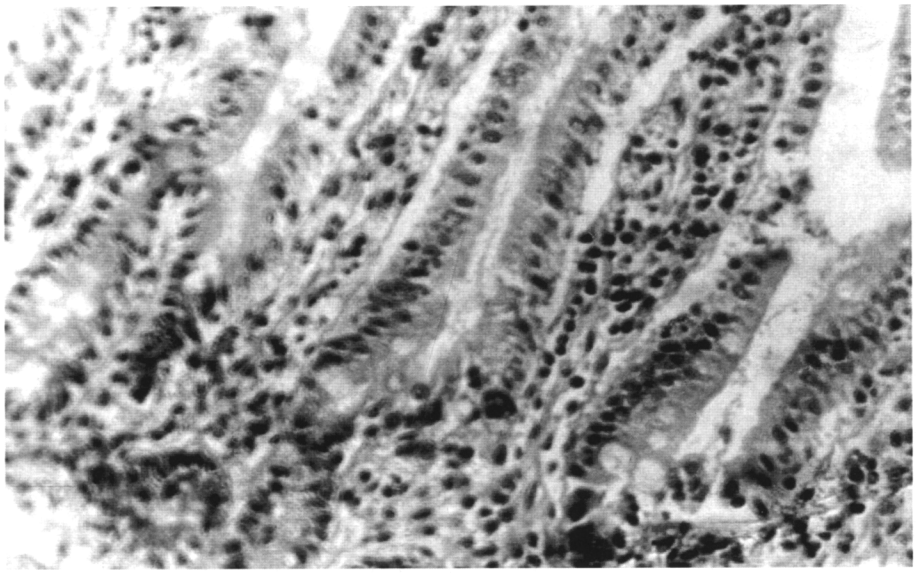
The importance of the immune cells associated to bronchus is well known (23). In a previous work (24) we demonstrated that short-term oral administration of LAB increased the IgA in the bronchus-associated lymphoid tissue (BALT). When we analyzed the effect of *L. casei* on the IgA+ cells in BALT, we observed a significant increase only until day 28 (Fig. 3). These results agree with the importance of local stimulation according to the compartmentalization theory (25). Intestinal *L. casei* stimulation would induce the increase in the IgA cycle, as described for other antigens, favoring mucosal immunity in BALT and mammary glands (26); however, in order to maintain the increase in the IgA cells, local stimulation is required. Another possibility is that down regulation is induced by the high level of IL-10 determined for day 28, which could induce a diminution in the proliferation and in the number of cells able to enter in the IgA cycle.

This paper is a preliminary study to demonstrate that the interaction between *L. casei* and the intestine, in conventional animals after long term cyclic oral administration, can induce different signals in the immune cells that lead to the activation of the mucosal immune response with cytokine release and an enhancement in the B cells producing IgA+, not only at the intestinal but also at the bronchus level, as well as peritoneal macrophage activation. This finding raises new questions concerning the immune mechanisms of the innate or adaptive immunity induced by non-pathogenic and non-commensal probiotic bacteria and the mechanisms for the down regulation to induce the oral tolerance necessary to maintain intestinal homeostasis.

This is a first step in acquiring knowledge on the safety of continuous consumption of a probiotic bacterium administered cyclically. Since different probiotic strains are known to have different immunological and microbiological properties, the modulation of the microflora and of the immune cells associated with the gut, using probiotics, has been shown to be important in different pathologies or infections; however, discrepancies in dose and duration of treatment make it difficult for clinicians to prescribe



1a



1b

Fig. 1. *Histological studies of the small intestine after long-term cyclic L. casei administration to mice. Tissues were prepared for histological evaluation using routine methods for paraffin inclusion according to Sainte Marie's technique. Four μm serial paraffin sections were cut and stained with haematoxylin-eosin. Fig. 1a shows the structure of the villi of the small intestine for NFM control mice. Fig. 1b shows a slight cellular infiltration in the lamina propria of the small intestine with a conserved histological structure of the villi in mice fed cyclically with L. casei for 28 days.*

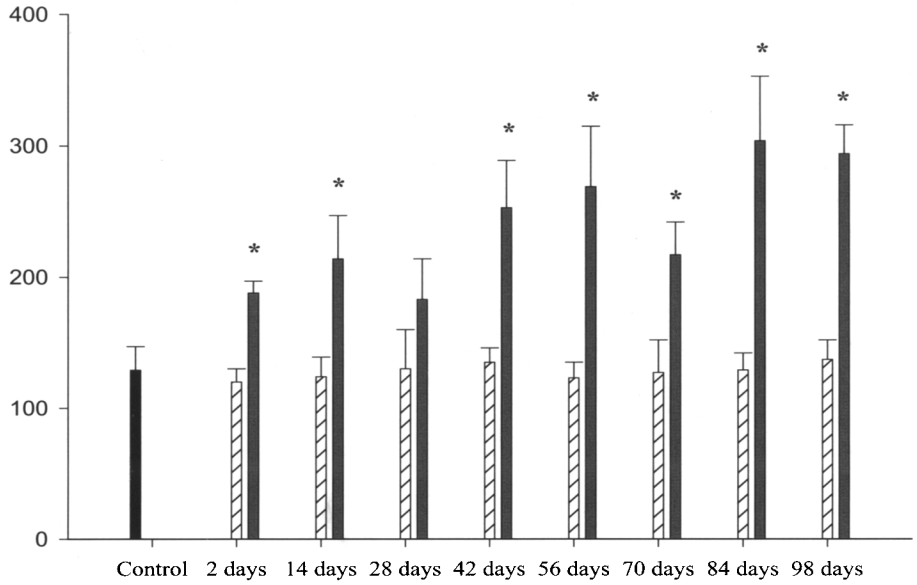


Fig. 2. *Effect of long term oral cyclic administration of L. casei on the IgA⁺ cells associated with the lamina propria of the small intestine. IgA⁺ cells were determined by direct immunofluorescence assays on histological sections from the small intestine of NFM control group (bars with diagonal lines) and test group (gray bars) fed cyclically with L. casei for 98 days. Black bar is for control group with no special feeding. Values are the mean of n=5 ± SD. Significant differences were calculated in relation to the NFM control group. * = P< 0.001.*

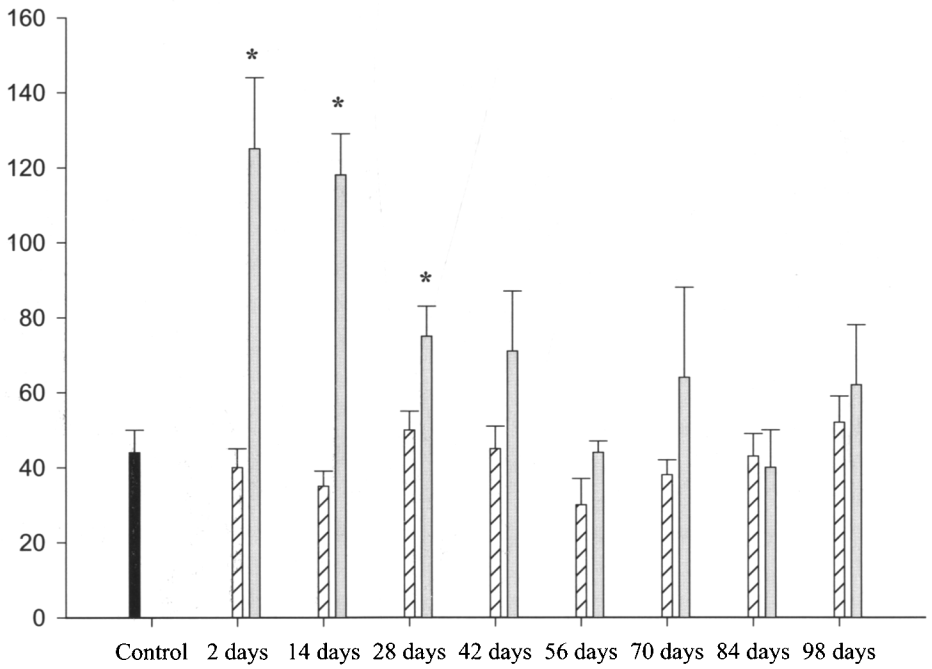


Fig. 3. *Effect of long term oral cyclic administration of L. casei on IgA+ secreting cells in bronchus. IgA⁺ cells were determined in histological slices from BALT by direct immunofluorescence assays in the different periods of time assayed for NFM control group (bar with diagonal lines) and test group (gray bars) fed cyclically with L. casei for 98 days. Black bar corresponds to the control group without special feeding. Values are the mean of n=5 ± SD. Significant differences were calculated in relation to the NFM control group. * = P< 0.001.*

a probiotic strain to improve mucosal and/or systemic immunity.

We have demonstrated the efficacy of a probiotic strain *L. casei* CRL 431 as an immunomodulator and an anti-inflammatory agent on the gut mucosal immune response, and its innocuity after long-term cyclic administration.

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REFERENCES

1. **Marteau P. and J.C. Rambaud.** 1993. Potential of using lactic acid bacteria for therapy and immunomodulation in man. FEMS. *Microbiol. Rev. 12:207.*
2. **Schiffrin E.J., D. Brassart, A.L. Servin, F. Rochat and A. Donnet-Hughes.** 1997. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. *Am. J. Clin. Nutr. 66: 515.*
3. **Schiffrin E.J., F. Rochat, H Link.Amster, J. Aeschlimann and A. Donnet-Hughes.** 1995. Immunomodulation of human blood cells following the ingestion of lactic acid bacteria. *J. Dairy Sci. 78: 491.*
4. **Vintiñi E., S. Alvarez, M. Medina, M. Médici, M.V. de Budeguer and G. Perdigón.** 2000. Gut mucosal immunostimulation by lactic acid bacteria. *Biocell 24:223.*
5. **Perdigón G., M.E. Bibas Bonet de Jorrat, S. Fontenla de Petrino and M. Rachid.** 1993. Antitumor activity of orally administered *Lactobacillus casei*: significance of its dose in the inhibition of a fibrosarcoma in mice. *Food & Agric. Immunol. 5:39.*
6. **Perdigón G., M. Medici, M.E. Bibas Bonet de Jorrat, M. Valverde and A. Pesce de Ruiz Holgado.** 1993. Immunomodulating effects of lactic acid bactéria on mucosal and tumoral immunity. *Int. J. Immunoth. 9:29.*
7. **Perdigón G., S. Alvarez and A.P. de Ruiz Holgado.** 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.*
8. **Perdigón G., S.Alvarez, N. Gobbato, M. de Budeguer and A.P. de Ruiz Holgado.** 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. Agr. Immunol. 7:283.*
9. **Alvarez S., N. Gobbato, E. Bru, A.P. de Ruiz Holgado and G. Perdigón.** 1998. Specific immunity induction at mucosal level by viable *Lactobacillus casei.* Perspective for oral vaccine development. *Food Agricul. Immunol. 10:79.*
10. **Perdigón G., C. Maldonado Galdeano, J.C. Valdez and M. Medici.** 2002. Interaction of lactic acid bacteria with the gut immune system. *Eur. J. Clin. Nutr. 56:21.*
11. **Sanders M.E.** 1993. Effects of consumption of lactic cultures on human health. *Adv. Food Nutr. Res. 17:67.*
12. **Mowat A.M.** 2003. Anatomical of tolerance and Immunity to intestinal antigens. *Nat. Rev. Immunology 3:331.*
13. **Perdigón, G., M. Medina, E. Vintiñi and J.C. Valdez.** 2000. Intestinal pathway of internalization of lactic acid bacteria and gut mucosal immunostimulation. *Int. J. Immunopathol. Pharmacol. 13:141.*
14. **Maldonado Galdeano C.M. and G. Perdigón.** 2004. Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. *J. Appl. Microbiol. 4:673.*
15. **Sainte-Marie G.** 1966. A paraffin embedding technique for studies employing immunofluorescence. *Proc. Sac. Exp. Biol. Med. 122:696.*
16. **Sartor R.B.** 1990. Role of intestinal microflora in initiation and perpetuation of inflammatory bowel disease. *Can. J. Gastroenterol. 4:271.*
17. **Moreau M.C. and G. Corthier.** 1988. Effect of the gastrointestinal microflora on induction and maintenance of oral tolerance to ovoalbumin in C_3H/He J mice. *Infect. Immun. 56:2766.*
18. **Weinstein P.D. and J.J. Cebra.** 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 environment. *J. Immunol. 147:4126.*

19. **Becker C. and M.F. Neurath.** 2004. Lamina propria dendritic cells mediate localized mucosal immuno response. *Mucosal Immunology Update 12:4.*
20. **Nagler-Anderson C.** 2001. Strategic defenses in the intestinal mucosa. *Nat. Rev. Immunol. 1:59.*
21. **Lamm M.E., J.G. Nedrud, C.S. Kaetzel and M.B. Mazanec.** 1996. New insights into epithelial cell function. In *Mucosal Immunity: Neutralization of intracellular pathogens and excretion of antigens by IgA.* M.F. Kagnoff and H. Kiyono, ed. Academic Press Inc San Diego California USA, p. 141.
22. **Bilsborough J. and J.L. Viney.** 2004. Directing the balance between intestinal homeostasis and active immunity: are gut plasmacytoid dendritic cell by to regulating intestinal immunity? *Mucosal Immunology Update 12:8.*
23. **Marquez M.G., G.A. Sosa and M.E. Roux.** 2000. Developmental study of immunocompetent cell in bronchus-associated lymphoid tissue (BALT) from Wistar rat. *Dev. Comp. Immunol. 24:386.*
24. **Perdigón G., S. Alvarez, M. Medina, E. Vintiñi and E. Roux.** 1999. Influence of the oral administration of lactic acid bacteria on IgA producing cells associated to bronchus. *Int. J. Immunopathol. Pharmacol. 12:97.*
25. **Brandtzaeg P. and R. Pabst.** 2004. Let`s go mucosal: communication on slippery ground. *Trends Immunol. 25:570.*
26. **de Moreno de LeBlanc A., C. Maldonado Galdeano, S. Chaves and G. Perdigón.** 2005. Oral administration of *L. casei* CRL 431 increases immunity in bronchus and mammary glands. *Eur. J. Inflamm. 3:25.*