A PROPOSAL MODEL: MECHANISMS OF IMMUNOMODULATION INDUCED BY PROBIOTIC BACTERIA

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Running title:Mechanisms of immunomodulation by probiotics

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The Intestinal Ecosystem

The mammalian microbiota comprises several hundred different bacterial species many of which have a beneficial effect on the host. For example they are involved in preventing colonization of the gut by pathogens and maintaining the gut mucosal immunity (85). The gut microbiota is more abundant in the large intestine of mammals, with densities rising to over $10^{11}$ organisms/g intestinal contents (84, 86). The number of bacterial cells in the entire gut exceeds the number of eukaryotic cells in the host, but under normal circumstance they coexist without any adverse effect on the host. The influence of the resident microflora on mucosal immune function and gut health has become an area of scientifical and clinical importance (22, 26). There is an active dialogue between the commensal microorganisms and the host mucosal immune system (21, 48). This crosstalk elicits different host responses to commensal and pathogenic bacteria. Commensal bacteria may even share molecular patterns recognized by toll-like receptors, which can recognize patterns associated mainly with pathogens. However, the mucosal immune system of the healthy intestine allows the persistence of this microbiota associated to the intestine and avoids immunological tolerance and maintaining the intestinal homeostasis. Now there is acceptance of the concept that oral tolerance is not generated by commensal intestinal bacteria; the host would ignore or fail to recognize the presence of indigenous microorganisms (49). The healthy host is able to elicit a good mucosal immune response against luminal antigens and to maintain a "physiological state of inflammation" in the gut, but it is also capable of responding to invading commensal organisms or pathogens. In the healthy host the penetration of the commensal bacteria is usually prevented by the barrier afforded by the intestinal epithelium and the immune cells associated with mucosa which are highly adapted to the presence of the normal microbiota (71). The signals sent by these microorganisms prevent their penetration and maintains them outside the intestinal tissue. If
the commensal microorganisms invade the host tissues, the innate immune mechanisms contribute to their rapid clearance, but when pathogens enter the intestine, innate and adaptative mechanisms are coordinately stimulated to respond the danger signals (38, 60). Although mucosal epithelial tissues form an efficient barrier that prevents the entrance of the environmental pathogens and the external antigens into the host internal milieu, mucosal tissues represent the main sites of infection by pathogens. Many attempts have been made to understand the gut immunomodulation by pathogenic bacteria, but not the mechanisms involved in the modulation of the gut immune system by commensal bacteria and by non-pathogenic microorganisms present in many foods included in the daily diet.

**Non-pathogenic probiotic bacteria**

The interest in the gut microflora, led to numerous investigations to demonstrate that there are beneficial and potentially harmful microorganisms in the intestine and that the one could be used to influence the activities of the other. These findings led to the “probiotic” concept, originally used to describe microbial feed supplements which stimulate the growth of farm animals. Now, the use of live microbes as dietary supplements has been extended to the human. Many definitions of probiotics have been published, starting from Fuller, who defined a probiotic “a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance” (25). A more recent one from FAO/WHO is: “Live microorganisms that when being administered in appropriate dose, they confer a benefit of health to the receiver.”

Some of the health benefits which have been claimed for probiotics are: improvement of the normal microflora (2), prevention of infectious diseases (3, 6, 9, 11, 19, 65, 83) and food allergies (51, 61), reduction of serum cholesterol (23, 77), anticarcinogenic activity (13, 17, 33, 35, 73), stabilizing the gut mucosal barrier (79), immune adjuvant properties (14, 20,
24, 28, 36, 40, 77, 80, 92) alleviation of intestinal bowel disease (IBD) symptoms (82) and improvement in the digestion of lactose in intolerant hosts (18, 42).

The genera most commonly used in probiotic preparations are *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus* and some fungal strains (58). Foods for human consumption containing mainly lactic acid bacteria they are included in fermented milks, cheeses, and fruit juices, wine and sausages. Single and mixed cultures of live microorganisms are used in probiotic preparations (4, 88).

The ability of probiotics to prevent or reverse several pathological conditions (27) by stimulating the immune system and all the scientific evidences in the immune system activation by probiotics indicates that the ability to generate an immune response should be included in the probiotic definition. Consequently we suggest probiotics should be defined as: “live microorganisms, that when includes in foods can influence the composition and activity of the gut microbiota, modulate the inflammatory response, improve the non-specific intestinal barrier, and reinforce or modulate the mucosal and the systemic immune response”. This definition ascribes to the probiotic microorganisms in the dietary supplement the potential for the prevention of infections, tumor growth or other systemic pathologies including effects in distant mucosal sites from the gut as in the bronchus (14, 69), mammary glands (15, 16) and urogenital tract (74, 91). However, for the best use of these microorganisms, the mechanisms by which they work should be understood. We believe that the selection of an appropriate probiotic strain for its inclusion in a probiotic preparation should be made on the basis of its capacity to induce an improved gut immune response without modification of the intestinal homeostasis. To achieve this task, probiotic strains should have the following properties: a) high cell viability, thus they must be resistant to low pH and bile acids; b) ability to persistence in the intestine even if, the probiotic strain cannot colonize the gut; continuous administration may be necessary c) the adhesion to the gut
epithelium to cancel the flushing effects of peristalsis. In this last aspect many relevant literature reports the adhesive property of the probiotic bacteria to epithelial cells by *in vitro* studies (5, 10, 29, 30, 87) d) They should be able to interact or to send signals to the immune cells associated to the gut. There are reports by *in vitro* assays, that show the activation of the immune cells after stimulation by probiotics (32, 54, 55, 81).

**What are the immune mechanisms induced by probiotic bacteria?**

The functioning of the gut mucosal immune system requires a complex network of signals with multiple interactions between commensal and foreign antigens and the eukaryotic cells. These include epithelial cells, macrophages, dendritic cells and other cells that belong to the non-specific barriers such mucus producer cells as goblet cells and Paneth cells which secrete antimicrobial peptides and produce cryptidins or defensins (76).

The mucosal epithelial cells are crucial in coordinating the defense mechanisms. They respond to environmental signals by releasing chemokines and cytokines that recruit the immune cells from both the innate and adaptative immune responses. These recruited immune cells can in turn act upon the epithelial cells stimulating the release of the cytokines. This response must not triggered by harmless intestinal commensal bacteria and the inflammatory response must be controlled. The particular characteristics of soluble, particulate antigens and pathogens will affect the gut immune response in relation to the way that they initiate the interaction with the immune system. At least three different routes exist for the uptake of luminal antigens: dendritic cells, specialized M cells from the Peyers patches and individual M cells found in the villous epithelium (39, 43). The anatomical location of the immune cells from the innate response (macrophages and dendritic cells) and the way by which these cells acquire antigens are crucial in determining the nature of the subsequent responses. Thus, the immune response induced can be the result of uptake of antigens by transepithelial sampling involving dendritic cells (75) or by dendritic cells.
present in the lamina propria of the intestine or by M cells from Peyer’s patches or from intestinal villous.

In the gut immune response induced by commensal bacteria, the antigen presentation from the luminal flora leads to the generation of large quantities of local IgA without induction of systemic immunity (56). The local S-IgA (secretory IgA) specific for the pathogen requires the interaction of phagocytic dendritic cells with T and B cells from the Peyer’s patches with the antigen presenting cells in isolated lymphoid follicles or in the mesenteric lymphoid node. The pathway of antigen internalization is crucial for the immune cell stimulation and the initiation of mucosal immune responses.

In the complex microenvironment of the gut, how can the transient population of non-pathogenic probiotic bacteria which may be unable to colonize the intestine, affect gut mucosal immunity? What kind of signals do they induce to act as oral adjuvants? Which kind of immune response do they elicit: innate or adaptative? How long do they have to remain in the gut to be effective? What is the quantity of these microorganisms needed to achieve the immunomodulatory capacity? Is the viability of the microorganisms a *sine qua non* condition required to induce such immunomodulation?

In order to survive probiotic bacteria entering by mouth must be resistant to pH, bile acid, proteolytic enzymes, antimicrobial peptides, intestinal peristalsis, luminal S-IgA blocking. The oral adjuvant capacity of some probiotic bacteria has been well demonstrated in our laboratory (90). How can this particulated antigen, without virulence factor, evade all the barriers of the host and up-or down-regulate the gut mucosal immune system? It is obvious that these non-pathogenic probiotic bacteria must interact with the epithelial cells and with the immune cells associated with the gut to start the network of immune signals. The increase in the number of IgA producing cells was the most remarkable property induced by probiotic microorganisms or by the fermented milk yoghurt (62, 68). The physiological role of IgA in
the mucosal surface is unquestionable (34, 45). The IgA+ B cells induced in the Peyer’s patches circulate through the mesenteric lymphatic node to enter into the blood via the thoracic duct and return to the intestinal mucosa repopulating mucosal distant sites such as the bronchus. Similar recirculation also occurs with intestinal T cells (70). Some probiotic microorganisms were also able to increase the IgA cycle and this effect was dose dependent (14, 67).

T independent IgA induction was also demonstrated; the cytokines TGFβ, IL-4 (50) and IL-2, IL-6 and IL-10 work in a synergistic way from other immune cells different from T cells, and can promote the switch from IgM to IgA expression (12, 44).

We demonstrated that some probiotic bacteria can act as adjuvants of the mucosal and systemic immune response (65, 68). The stimulation with probiotic bacteria induced signals on epithelial and immune cells that evoke different patterns of cytokine in the intestine (53, 64, 89), depending on the dose administered (table 1) as has also shown by Massen et. al (47). The quantity of these microorganisms to achieve the adjuvant effect in the mucosal or systemic immune response was 1x10^8 – 1x10^9 C.F.U./day (68, 90).

In the analyzes of the profile of cytokine induced by some lactic acid bacteria, we observed the most remarkable effect was the increase in the TNFα, IFNγ and in the regulatory cytokine IL-10 for all the probiotic strains assayed. This effect was obtained without increasing the inflammatory response and only slight increasing the cellularity was determined. However the induction of TNFα by the probiotic bacteria would be necessary to initiate the cross talk between the immune cells associated with the lamina propria and the intestinal epithelial cells. IFNγ would also play a physiological role; it was demonstrated that this cytokine is necessary for the maturation of some immune cells such as dendritic cells and also controls their cellular proliferation at the intestinal level (78).
It was previously thought that to have an effect on the immune system, the probiotic strains must remain viable. We demonstrated (52) that this fact is true only for some strains. For *Lactobacillus delbrueckii* subsp. *bulgaricus*, viability was not necessary for the induction of positive cells producing cytokines; although the number of positive cells was comparatively lower than the number obtained with the viable *L. delbrueckii* subsp. *bulgaricus*. The viability was critical for determining the time of residence in the gut with differences between viable and non-viable probiotic bacteria administration; non-viable bacteria were cleared more rapidly. We also demonstrated that the probiotic bacteria must remain in the gut to be effective at least 48 – 72 hs that is the time require for any particulate antigen to induce gut immunostimulation (52, 63). This fact is a very important finding meaning the importance of the daily administration in the dose established for each probiotic bacteria to have adjuvant effect without the induction of oral tolerance.

We and other workers have demonstrated that the probiotic microorganisms are able to induce a gut mucosal immune response (41, 63), which requires the bacteria to interact with the epithelial and immune cells in the gut to induce the network of signals involved in an immune response.

Probiotic bacteria may arrive in the intestine along routes which correspond with the different pathways for the internalization of the antigens. These bacteria (as whole cells or as antigenic fragments) must interact with the M cells in the Peyer’s patches, with gut epithelial cells and with the associated immune cells. After the contact with these cells, the release of cytokines is induced to up- or down-regulate the immune response.

How these non-pathogenic bacteria interact with the intestinal epithelial cells? What kind of signals do they induce in the immune cells in order to initiate the gut response?

Mucosal epithelial cells form an efficient barrier which prevents antigens of environmental pathogens gaining access to the host milieu. Flagellated microorganisms, including
commensals, trigger epithelial homeostatic chemokine responses that recruit immune cells of
the innate immune system to the epithelium and lamina propria of the intestine to link the
innate or/and the adaptative immune response (78). It has also been shown that commensal
bacteria can activate toll-like receptors (TLRs) signal (37). Although, the precise location of
these receptors in the intestinal epithelial cells (apical or/and basolateral) is controversial (8),
TLR signals are essential, not only for response to pathogens (59), but also to maintain the
intestinal barrier function (72).
By in vivo studies in mice we demonstrated the pathway of internalization of the following
probiotic bacteria: Lactobacillus bulgaricus CRL 423, L. casei CRL 431, L. acidophilus CRL
728, Streptococcus thermophilus CRL 412 (66, 67). We determined the probiotic bacteria in
Peyer’s patches, in the lamina propria of the villi of the small intestine and in the nodule of
the large intestine. We also demonstrated that Lactobacillus casei CRL 431 interacts with the
epithelial cells of the small intestine and that their fragments can internalize and activate the
intestinal epithelial cells (52, 63). It was also shown by in vitro and ex vivo studies in a
primary culture of intestinal epithelial cells from conventional animals (89) that probiotic
bacteria interact with these cells and induce release of IL-6 but not IL-1 and IL-10. This study
also demonstrated that TLR-2 is involved in this interaction and could be responsible of the
signals induced for IL-6 release by epithelial cells (Table 2 and figure 1), which would be
other source of IL-6 induced by probiotic bacteria, than the immune cells associated to the
gut.
We showed that probiotic bacteria could be also internalized through M cells into the Peyer’s
patches or villi or may be sampled by dendritic cells as whole cells or their antigenic
fragments (52, 63, 66, 67). These may be captured by other dendritic cells or macrophages
associated with the lamina propria to increase the signals to the epithelial cells and/or other
immune cells. There are scientifical evidences that the uptake of non-pathogenic bacteria or
their fragments by macrophages or dendritic cells in the lamina propria is possible through direct sampling of luminal antigen for dendritic cells through TLRs and CD-206 mannose receptor (1, 46). These bacteria can be cleared or transported to the mesenteric lymphatic node in which they interact with T and B cells to induce specific mucosal IgA or suppress T cells (57).

When probiotic bacteria labelled with FITC were administered to mice, we found fluorescent cells at different levels of the intestine in Peyer’s patches, lamina propria of the villi and nodules of the large intestine (figure 2 A, B, C) (52, 66). The possible pathway of internalization to the villi of the whole bacteria could be through of the M cells present in the villi (39). For the bacterial particles TLR-2 or CD-206 receptor would be involved as was demonstrated, after *L. casei* CRL 431 administration (53, 89), there was a remarkable increase of both of these receptors in the immune cells associated to lamina propria or in cells isolated from Peyer’s patches. The IgA+ cells in lamina propria of the small intestine were increased for different lactic acid bacteria such as *L. acidophilus, L. bulgaricus, S. thermophilus* (63, 67, 68, 90). Specific IgA against the probiotic bacteria, and modifications in the number of CD4+ population were not found (53, 90). These findings would show that antigenic presentation with production of specific antibodies would not be induced. In previous studies in physiological conditions (healthy animals) we observed that the administration with different probiotic bacteria did not increase CD4+ or CD8+ population (53, 90). The results obtained for positive cells for cytokine release analyzed in isolated immune cells from Peyer’s patches showed that the adherent population (macrophages and dendritic cells) had a more relevant effect on cytokine production (64).

Even though we cannot ignore that other mucosal immune mechanisms such as the Th1 cells response can be modulated by probiotic bacteria as was demonstrated by other authors in pathological process such as allergy (36), inflammatory bowel disease (7, 8, 17) or colon.
cancer (13, 15, 16). Our previous scientific evidences in physiological conditions led us to suggest that the probiotic bacteria interact with the epithelial cells and preferentially with the immune cells from the innate immune system reinforcing this barrier (52, 53, 63, 89). When they interact with cells from Peyer’s patches they can induce an increase of the IgA cycle as was demonstrated in our laboratory (14, 69). According these previous studies where we demonstrated: 1) the epithelial interaction of the probiotic bacteria; 2) the pathway of internalization of probiotics to the gut; 3) the signals induced to the immune cells associated to the intestine by an increase in the cytokine production and an increase in the number of IgA secreting cells; 4) the increase of IgA secreting cells in other distant mucosal sites such as bronchus and mammary glands, as consequence of gut stimulation by probiotic bacteria. We suggested that in physiological conditions, probiotic bacteria can act as mucosal and systemic adjuvants. This last effect would be mediated by the network of cytokines induced after probiotic stimulation. In our opinion, the most important signals induced by probiotic bacteria included in daily food would be mediated through the immune cells involved in the innate immune response. The model proposal by probiotic interaction and gut immune activation in our opinion are shown in figure 3 A and B and figure 4.

In conclusion, we demonstrated for the probiotic microorganisms that the most important mechanisms involved in the gut immune stimulation are the clonal expansion of LB-IgA+ and on the innate immune response. The magnitude of such stimulation did not enhance the inflammatory immune response. They induced up- or down-regulation of the innate response in order to maintain the intestinal homeostasis. Even when T cell population was not modified in the lamina propria of the intestine, we can not exclude T cells activation as a source of the cytokines detected.

More studies concerning the different signals induced by probiotic microorganisms involved in the activation of the immune cells, through of the distinct receptors are necessary. These
researches will allow to establish the big difference among the signals induced by pathogens (beside of their virulence factors) that use similar receptors, commensal and non-commensal probiotic bacteria, to induce inflammatory immune response or immunomodulatory effects. The proposed model on the mechanisms induced by probiotic bacteria from our studies show at least in part the scientific basis of the way in which the probiotics work. This knowledge would be also useful to influence the gut immune system in pathological conditions.

Acknowledgement:

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REFERENCE


Table 1: Effect administration of the lactic acid bacteria on the number of IgA and cytokine producing cells in the lamina propria of the small intestine.

<table>
<thead>
<tr>
<th>Lactic acid bacteria</th>
<th>Feeding period</th>
<th>Cytokines</th>
<th>IgA</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>TNFα</td>
<td>IFNγ</td>
</tr>
<tr>
<td><em>L. casei</em> CRL 431</td>
<td>2 days</td>
<td>90 ± 8*</td>
<td>124 ± 15*</td>
</tr>
<tr>
<td></td>
<td>5 days</td>
<td>74 ± 10*</td>
<td>116 ± 18*</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>52 ± 7</td>
<td>85 ± 19*</td>
</tr>
<tr>
<td><em>L. delbrueckii</em> subsp. <em>bulgaricus</em> CRL 423</td>
<td>2 days</td>
<td>79 ± 6*</td>
<td>59 ± 22*</td>
</tr>
<tr>
<td></td>
<td>5 days</td>
<td>59 ± 11</td>
<td>72 ± 18*</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>43 ± 12</td>
<td>209 ± 34*</td>
</tr>
<tr>
<td><em>L. acidophilus</em> CRL 724</td>
<td>2 days</td>
<td>52 ± 7</td>
<td>51 ± 25*</td>
</tr>
<tr>
<td></td>
<td>5 days</td>
<td>51 ± 9</td>
<td>73 ± 11*</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>22 ± 11</td>
<td>64 ± 6*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>24 ± 4</td>
<td>17 ± 6</td>
</tr>
</tbody>
</table>

The cytokine producing cells and the IgA secreting cells were determined on histological slices from the small intestine of BALB/c mice by an immunofluorescence test. The animals were feed in the drinking water with LAB (1×10⁸ UFC/ml/day) during 2, 5 or 7 consecutive days. *L. casei* and *Lactobacillus acidophilus* were isolated from human faeces and *Lactobacillus delbrueckii* subsp. *bulgaricus* from yogurt. The animals received 2.5 or 3 ml/day. Values are the mean of N = 3 ± SD. * = significant differences between test and untreated control, P < 0.001.
Table 2. IL-6 production by small intestine epithelial cells (SIEC) isolated from conventional animals and challenged with different concentrations of viable cultures of *L. casei* CRL 431 and *L. helveticus* R389

<table>
<thead>
<tr>
<th>A</th>
<th>In vitro IL-6 production (pg/ml) by SIEC</th>
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<tr>
<td></td>
<td>10⁸ CFU/ml</td>
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<tr>
<td><em>L. casei</em> CRL431</td>
<td>245±10*</td>
</tr>
<tr>
<td><em>L. helveticus</em> R389</td>
<td>190±20*</td>
</tr>
<tr>
<td>LPS (0.1 µg/ml)</td>
<td>878±22*</td>
</tr>
<tr>
<td>Control</td>
<td>310±11</td>
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<table>
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<tr>
<th>B</th>
<th>Ex vivo IL-6 production (pg/ml) by SIEC</th>
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<tr>
<td></td>
<td>Days of feeding</td>
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<tr>
<td></td>
<td>2d</td>
</tr>
<tr>
<td><em>L. casei</em> CRL431</td>
<td>380±82*</td>
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<tr>
<td><em>L. helveticus</em> R389</td>
<td>499±85</td>
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<tr>
<td>Control</td>
<td>494±47</td>
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<table>
<thead>
<tr>
<th>C</th>
<th>In vitro IL-6 production (pg/ml) by SIEC</th>
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<tr>
<td></td>
<td>non-treated</td>
</tr>
<tr>
<td></td>
<td>with anti-TLRs</td>
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<tr>
<td><em>L. casei</em> CRL431</td>
<td>531±60*</td>
</tr>
<tr>
<td><em>L. helveticus</em> R389</td>
<td>641±75*</td>
</tr>
<tr>
<td>LPS (0.1 µg/ml)</td>
<td>863±80*</td>
</tr>
<tr>
<td>Control</td>
<td>399±15</td>
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</table>

(A) *In vitro* assay. IL-6 released by SIEC from BALB/c mice when the cells were challenged with different concentration of the bacterial strains, of LPS or without challenge (control). (B) *Ex vivo* assay. IL-6 production by SIEC isolated from BALB/c mice that received viable culture (1x10⁸ C.F.U / ml/day) of *L. casei* isolated from human faeces and *L. helveticus* isolated from cheese, during different period of time (2, 5 or 7 days). (C) IL-6 production by SIEC treated or not with anti-TLR2 and anti-TLR4 antibodies before they were stimulated with the bacterial strains or with LPS. LPS was used as a positive control.

* Significantly different from the corresponding control value (P< 0.05).
FIG. 2
FIG. 3
FIG. 4
FIGURE LEGENDS

Figure 1
The scanning electron micrograph show the interaction of *Lactobacillus helveticus* R389 with the intestinal epithelial cells (IEC) obtained from the small intestine of BALB/c mice. The epithelial cells were recovered from the small intestine after digestion with collagenase in adequate culture medium. *Lactobacillus helveticus* suspension (10^{12} CFU/ml) was added to IEC isolated. After bacterium contact with IEC during 2 hours, the samples were processed for scanning electron micrograph. Magnification 6000X.

Figure 2
Histological slices of intestine of BALB/c mice that show the pathway of internalization to the gut by LAB. Animals received FITC-labeled lactic acid bacteria (1x10^{8} UFC/ml) by intragastric intubation
A)- Fluorescence in Peyer’s patches of the small intestine of mice after 5 min. of labeled *Lactobacillus delbrueckii* subsp. *bulgaricus* CRL 423 (isolated from yogurt) administration.
B)- Fluorescence in lamina propria of the small intestine of mice that received labeled *Lactobacillus casei* CRL 431 isolated from human faeces. The samples were processed after 5 min of lactobacilli administration.
C)- Fluorescence in the nodule and crypts of the large intestine of mice after 10 min of labeled *Lactobacillus acidophilus* CRL 724 isolated from faeces.

Figure 3
Local immune response in the gut induced by the interaction between probiotic bacteria and the epithelial and the immune cells associated to the lamina propria of the small intestine. Activation of the innate immune response.
There would be different pathways of internalization for the probiotic bacteria present in the lumen of small intestine: The M cell (MC) associated to the epithelium, the epithelial cell (EC) and the interdigitant dendritic cells (DC) able to sample bacteria. After the interaction with the epithelial cells, probiotic bacteria or their fragments are internalized, the first cells that would interact with them are the antigen presented cells (APC), macrophages and/or dendritic cells associated to the lamina propria of the gut. The interaction with epithelial cells induces IL-6 release. Macrophages and dendritic cells phagocyte the probiotic bacterium or their fragments and they are induced to produce cytokines as TNFα and IFNγ that increase epithelial cells stimulation and initiate the cross talk between all the associated immune cells. Mast cell would be also stimulated to produce IL-4. Other cytokines such as IL-10, and IL-6 are also produced to enhance the cytokine network of signals. The ingested bacteria or their particles could also be eliminated by phagocytosis clearance.
The IL-6 would favour the clonal expansion of IgA B-lymphocytes increasing the number of IgA producing cells and the passage of them to plasmatic cells in the lamina propria of the gut. The IL-6 together IL-4 and TGF-β (not determined in our studies) can induce the T independent switch from IgM to IgA in the surface of B cells and to promote by this way the increase in the number of B cells IgA+ in the lamina propria of the gut.
CE= intestinal epithelial cells, MC= M cells, CD=dendritic cells, MQ=macrophages, LT=T lymphocytes, LB=B lymphocytes, MS=mast cells, PC=plasmatic cells.

Figure 4.
Systemic immune response induced by probiotic bacteria after interaction with the immune cells of the Peyer’s patches.
In the Peyer’s patches the probiotic bacteria or their fragments are internalized by M cells or by paracellular way through the follicle associated epithelial cell (FAE) of the Peyer’s patches. After that the bacteria or their particles interact with the macrophages and dendritic cells which are activated to produce cytokines. As consequence of the bacterial stimulation to the immune cells in this inductor site of the immune response, the cytokine production is enhanced as well as the switch from IgM to IgA B cells. IL-10, IL-6, IL-4 TGFβ from the immune cells could also promote this T independent switch. Probiotic stimulation can induce the IgA cycle increasing the number of IgA+ cells in mucosal sites distant to the intestine. The IgA+ cells migrate to the mesenteric lymphoid node and then via thoracic duct to the circulation arriving to bronchus and mammary glands. The cytokines release by probiotic stimulation in Peyer’s patches, are the biological messengers of the complex network of signals to activate the systemic immune response.

DC=dendritic cells, MQ=macrophages cells, APC= antigenic presented cells, TL= T lymphocytes, BL= B lymphocytes