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Meccanismi della modulazione immunologica dei probiotici

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Meccanismi della modulazione immunitaria

Nelle diapositive seguenti:

- **I probiotici stimolano il sistema immunitario?**
- **Le componenti della cellula batterica coinvolte nella stimolazione**
- **Le sostanze secrete immunostimolanti**

Probiotics and the immune system

- **Modulation** of the immune system (GALT but not only…) **is well established**
- Modulation is **strain dependent** (sometimes opposite actions within the same species)

Basic facts

- **The first steps:**

a) mice immunized intraperitoneally (Bloksma et al, 1979)

b) human orally fed during a Salmonella vaccine treatment (Schiffrin et al,1995)

- **Two second steps**:

a) support vaccination

b) delivery vectors (GMM)

The beginning

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United States Patent **5,603,930**
Brassart , et al. **February 18, 1997**

Lactobacillus johnsonii CNCM I-1225

Abstract

Lactobacillus johnsonii strain CNCM I-1225 adheres to Caco-2 cells and inhibits adhesion thereto by enterovirulent and enteroinvasive pathogens.

Human volunteers (response to a vaccine)

16 Healthy human volunteers (test group) followed the following eating program: for two weeks (weeks 1 and 2), normal diet excluding any fermented product; for the following three weeks (weeks 3, 4 and 5), mixed diet of three

125 ml yohurts per day, the yogurts having been prepared by fermentation of a milk with a commercial culture of S. thermophilus and Bifidobacterium bifidus to which the L. johnsonii strain CNCM I-1225--present in this yogurt--was added in a quantity of 10.sup.7 to 10.sup.8 cfu/ml; and for another two weeks (weeks 6 and 7) normal diet excluding any fermented products.

14 Healthy human volunteers (control group) simultaneously followed an eating program consisting of a normal diet excluding any fermented product.

A vivotive oral vaccine (Salmonella typhi Ty21a) marketed by Berna SA was administered to the volunteers of the two groups in accordance with the manufacturer's instructions on days 1, 3 and 5 of week 4.

Blood samples were taken from all the volunteers 3 days after the beginning of week 3 and 1 day and 10 days after the end of week 5.

Determination of the concentration of the specific IgA's of the immune response to the antigenic lipopolysaccharides (LPS) of Salmonella typhi was carried out by the ELISA method.

It was found that the increase in the concentration of the specific IgA's observed fifteen days after vaccination in relation to the concentration observed nine days before vaccination is significant in the two groups (p value >0.001).

However, if ranges of increase factors <2; >2 and <3; >3 and <4; >4 are taken into consideration, respective distributions are observed in these ranges of 1, 6, 3 and 6 volunteers for the test group against 8, 3, 0 and 3 volunteers for the control group. In other words, the increase factors are significantly higher in the test group than in the control group (p value=0.04).

European Food Safety Authority

EFSA Journal 2015;XXX:YYYY

1 **DRAFT SCIENTIFIC OPINION**

2 Guidance on the scientific requirements for health claims related to the 3 gastro-intestinal tract, the immune system, and defence against pathogenic 4 microorganisms [1]

5 **EFSA Panel on Dietetic Products, Nutrition and Allergies**[2, 3]

6 European Food Safety Authority (EFSA), Parma, Italy

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efsa European Food Safety Authority

EFSA Journal 2015;XXX:YYYY

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653 effect (e.g. through the activation of the immune system), but cannot be used alone for the scientific
654 substantiation of these claims.

655 Vaccination confers immunity to certain infectious diseases. Even if a strict correlation between titres
656 in response to vaccination and protection against infection is not always evident, cut-off values of
657 antibody-titres in response to vaccination indicating protection have been established for many
658 vaccines. Higher responses to vaccination (as measured by increased numbers of individuals attaining
659 protective levels of antibody titres) are appropriate outcome variables for the scientific substantiation
660 of claims related to the immune defence against pathogens.

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Adjuvants in flu vaccine in brief

- Species and strains used:
- *L.casei* NCC NCC 2461 and *L.paracasei* DN-114 001 for elderly
- *L-rhamnosus* ATCC53103 and *L. fermentum* CECT5716 for adults
- Daily dosage from 1 billion to 10 billions
- Treatment from 28 days up to 13 weeks

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Adjuvants in flu vaccine in brief

Adjuvant effects in combination with vaccine consisting of attenuated viruses.

Virus: flu viruses H1N1, H3N2 and B in adults and elderly; Hepatitis in infants

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- About 400 elderly and 50 adults in the treatment groups;
- 4 different lactobacilli used, all belonging to the heterofermentative taxonomic groups
- Different outcomes; all trials overall positive but (apparently) with a different activity against different viral strains
- Strain specificity or different protocol design?

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Adjuvants in other vaccinacination

- Specific antibody responses to infant Hepatitis B vaccination (Soh et al,2010); two strains

(*B. longum* BL999 and *L. rhamnosus* LPR)

Positive outcomes in the case of a single dose, not in a schedule of 3 doses

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Adjuvants in other vaccinacination

- Inactivated cholera in children: one study in Bangladesh with *B.breve* BBG-01 (Matsuda et, 2011): no adjuvant effect in children
- Inactivated cholera in adults: 6 out of 7 strains assayed singularly showed faster immune response measured with by means of serum immunoglobulin but overall vaccination was not influenced.(Paineau et al, 2008)

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Adjuvants in other vaccinacination

- *L.acidophilus* LAVRI-A1 at a daily dosage of $3X10^9$ CFU/day x 6 months (Taylor et al, 2006). Tetanus (TT) and *Staphylococcus*
- enterotoxin B (SEB) vaccines used.
- “In summary, although we did not see any consistent effects on allergen-specific responses, our study suggests that probiotics may have immunomodulatory effects on vaccine responses.”

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Adjuvants in other vaccinacination

- The effects of probiotics on antibody responses to diphtheria, tetanus and *Haemophilus influenzae* vaccines in 6-month-old infants were mesured by Kukkonen et al in 2006.
- Probiotics used were four strains (LGG*, L.rhamnosus* LC705, B. breve Bbi99 and P. freudenreichii

  ssp. shermanii JS)
- Differences in concentrations of anti-diphtheria or anti-tetanus IgG were not significant.

  In the probiotic group, the geometric mean

  Hib IgG concentration tended to be higher, but

  the difference was not significant

Adjuvants in other vaccination

Table 2. IgG antibody concentrations against diphtheria, tetanus, and *Haemophilus influenzae* type b (Hib) in vaccinated 6-month-old infants receiving probiotics, or a placebo

	Probiotic	Placebo	Ratio probiotic/placebo (95% CI)	p-value
Diphtheria (IU/ml)	0.38 (0.14–0.78) (37)	0.47 (0.19–1.40) (37)	0.80 (0.45–1.43)	0.449
Tetanus (IU/ml)	1.01 (0.47–1.49) (37)	0.81 (0.56–1.39) (37)	1.23 (0.82–1.86)	0.310
Hib (μg/ml)	0.75 (0.15–2.71) (32)	0.40 (0.15–0.92) (29)	1.87 (0.96–3.64)	0.064

Results are expressed as geometric mean and inter-quartile range in parenthesis. Number of infants is in brackets. Comparisons between study groups by the ratio probiotic/placebo with a 95% CI.

The probiotic mixture was formed by 4 strains;
The clear final effect was vaccine-related

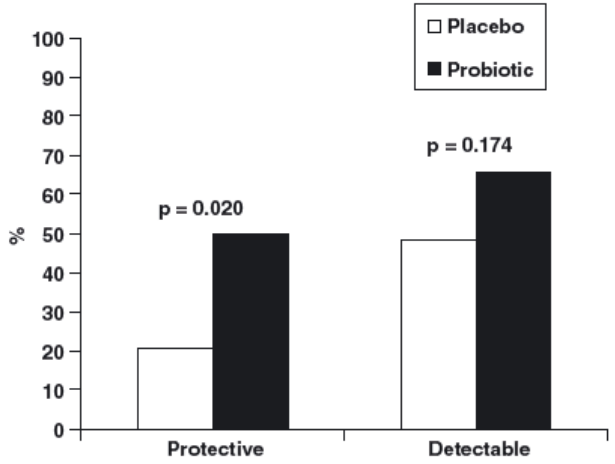

Fig. 2. Prevalence of protective (≥1 μg/ml) and detectable (≥0.3 μg/ml) *Haemophilus influenzae* type b IgG antibody concentrations after primary Hib conjugate vaccine dose in infants receiving probiotics (n = 32), or placebo (n = 29).

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Meccanismi della modulazione immunitaria

VIEWPOINT

The impact of probiotics and prebiotics on the immune system

Todd R. Klaenhammer, Michiel Kleerebezem, Matthias Volkmar Kopp and Maria Rescigno

Abstract | Probiotics and prebiotics are increasingly being added to foodstuffs with claims of health benefits. Probiotics are live microorganisms that are thought to have beneficial effects on the host, whereas prebiotics are ingredients that stimulate the growth and/or function of beneficial intestinal microorganisms. But can these products directly modulate immune function and influence inflammatory diseases? Here, *Nature Reviews Immunology* asks four experts to discuss these issues and provide their thoughts on the future application of probiotics as a disease therapy.

Do probiotics and prebiotics modulate immune function? And if so, how?

Klaenhammer et al,2009

Meccanismi della modulazione immunitaria

Do probiotics and prebiotics modulate immune function? And if so, how?

Si, con una azione ceppo specifica e attraverso due categorie di meccanismi:

1. Interazione fra GALT e strutture di superficie dei batteri

2. Interazione con sostanze secrete e assorbibili

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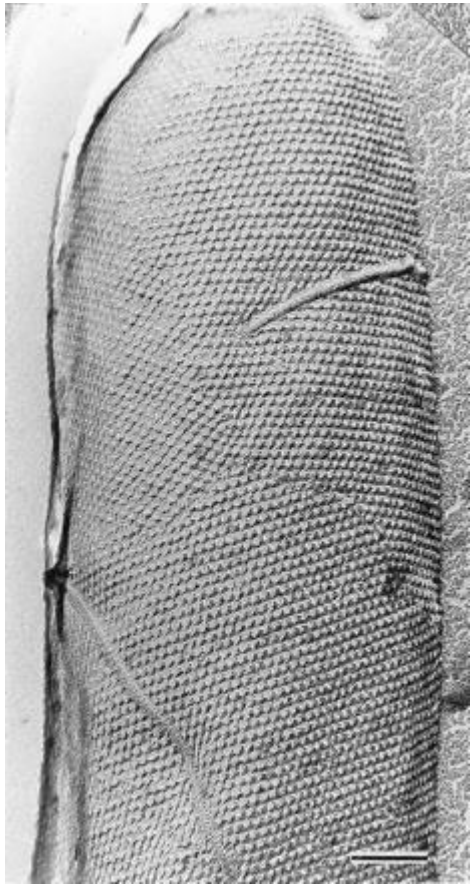
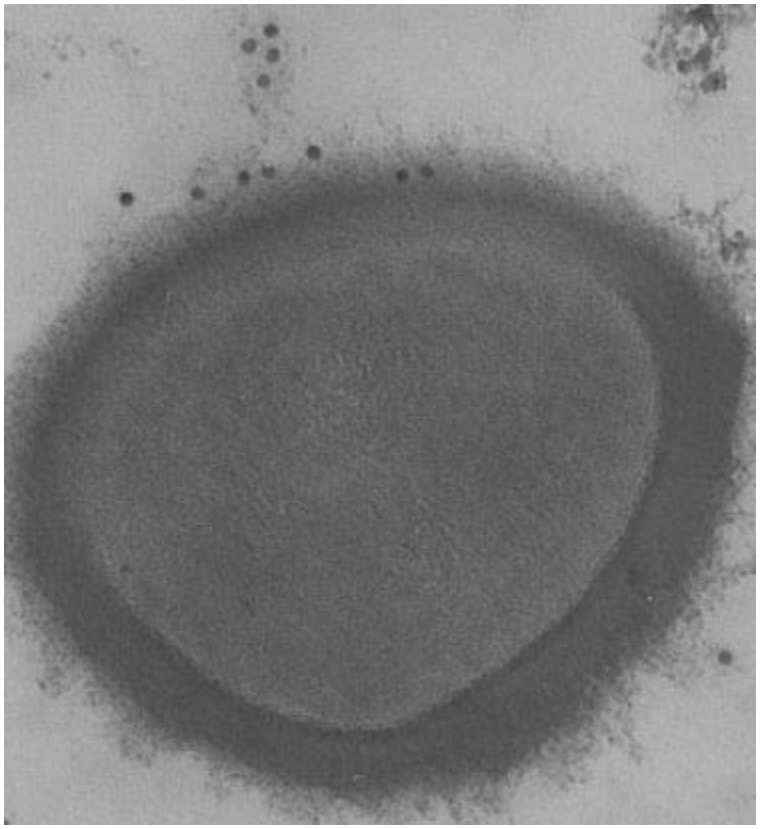
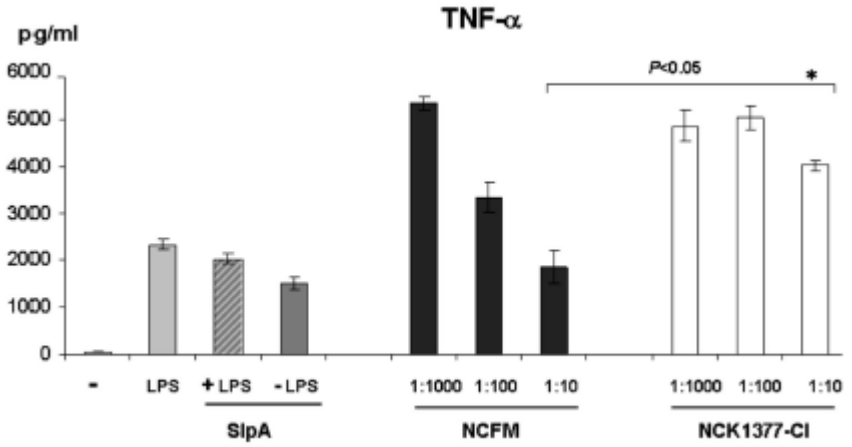
S-layer come struttura di superficie





S-layer in *L.acidophilus* NCFM

Dendritic cells (DCs) are antigen-presenting cells that play an essential role in mucosal tolerance. They regularly encounter beneficial intestinal bacteria, but the nature of these cellular contacts and the immune responses elicited by the bacteria are not entirely elucidated. Here, we examined the interactions of *Lactobacillus acidophilus* NCFM and its cell surface compounds with DCs. *L. acidophilus* NCFM attached to DCs and induced a concentration-dependent production of IL-10, and low IL-12p70. We further demonstrated that the bacterium binds to DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN), a DC- specific receptor. To identify the DC-SIGN ligand present on the bacterium, we took advantage of a generated array of *L. acidophilus* NCFM mutants. A knockout mutant of *L. acidophilus* NCFM lacking the surface (S) layer A protein (SlpA) was significantly reduced in binding to DC-SIGN. This mutant incurred a chromosomal inversion leading to dominant expression of a second S layer protein, SlpB. In the SlpB-dominant strain, the nature of the interaction of this bacterium with DCs changed dramatically. Higher concentrations of proinflammatory cytokines such as IL-12p70, TNFα, and IL-1β were produced by DCs interacting with the SlpB-dominant strain compared with the parent NCFM strain. Unlike the SlpA-knockout mutant, T cells primed with *L. acidophilus* NCFM stimulated DCs produced more IL-4. The SlpA–DC-SIGN interaction was further confirmed as purified SlpA protein ligated directly to the DC-SIGN. In conclusion, the major S layer protein, SlpA, of *L. acidophilus* NCFM is the first probiotic bacterial DC-SIGN ligand identified that is functionally involved in the modulation of DCs and T cells functions.



Kostantinov et al, 2008

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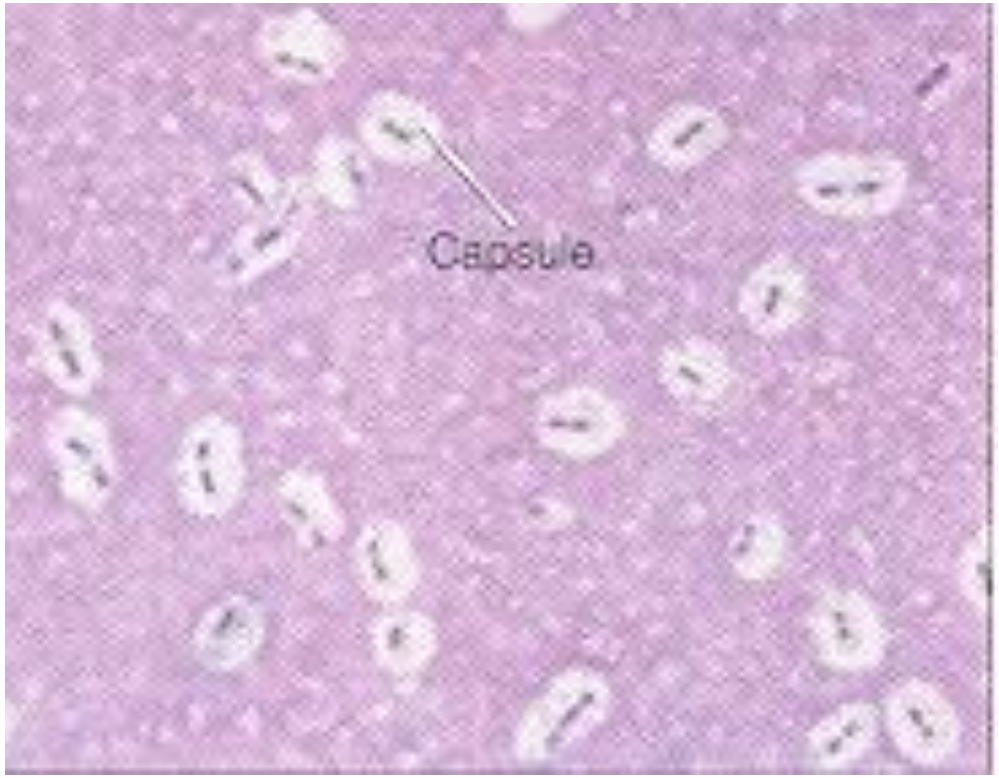
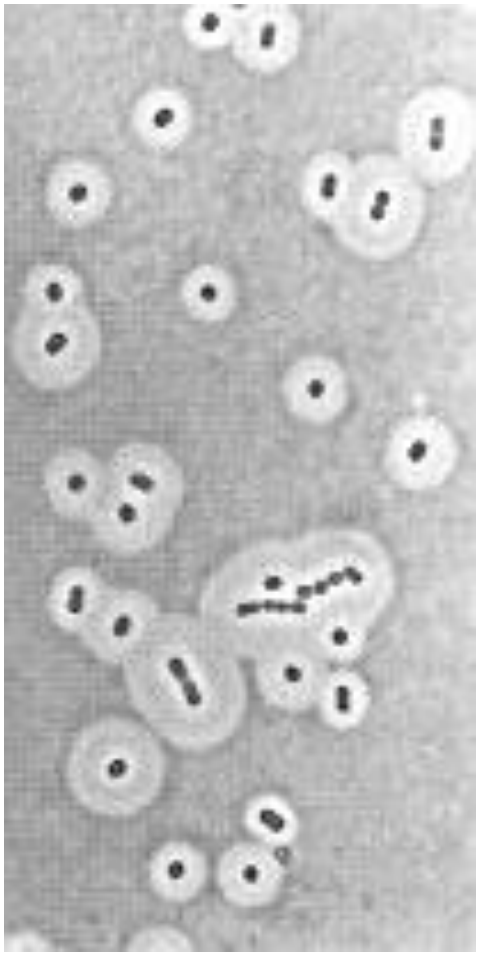
Le capsule polisaccaridiche





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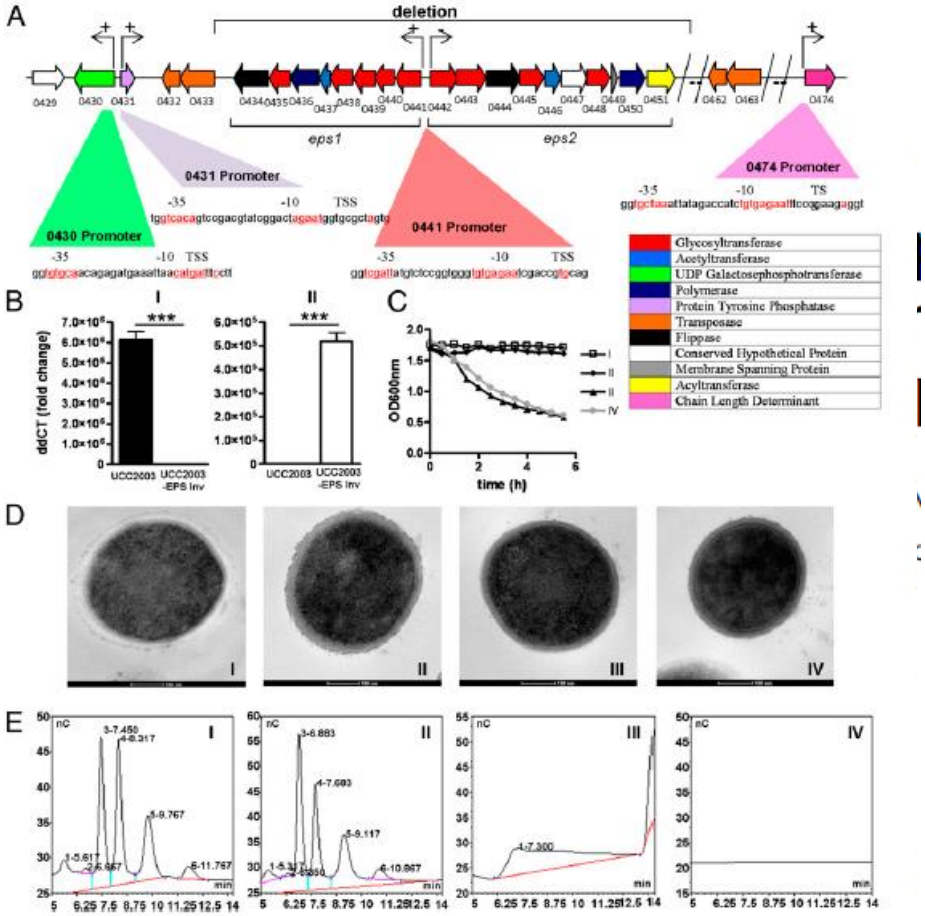
Le capsule polisaccaridiche



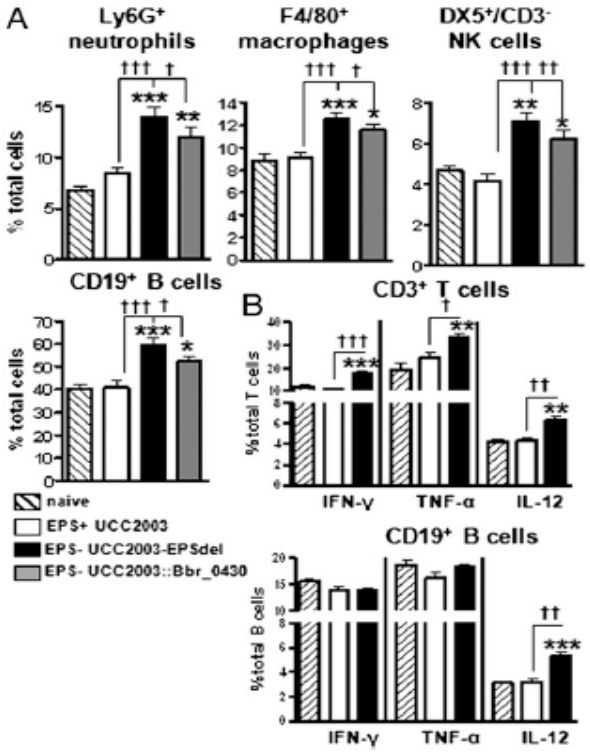


Fig. 3. *B. breve* surface EPS modulates recruitment and cytokine profile of immune cell populations in mice. (*A*) Cells were isolated from spleens of BALB/c mice 31 d after initial treatment, stained with fluorochrome-labeled mAb, and analyzed by flow cytometry. Columns represent the mean percentage ± SD of at least eight mice from two independent experiments. (*B*) Isolated cells were stimulated for 6 h with BD Leukocyte Activation Mixture plus GolgiPlug in vitro, stained with surface mAb to determine $CD3^+$ and $CD19^+$ populations, and then permeabilized and stained with anticytokine fluorochrome-labeled mAb. Data represent percent of cytokine-positive cells out of total specific cell population ± SD. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ between naïve and *B. breve*-treated mice; $^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.01$, and $^{\dagger\dagger\dagger}P < 0.001$ between EPS^+ and EPS^- using one-way ANOVA followed by Bonferroni's multiple comparison test.

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Le sostanze secrete immunostimolanti

Comparison of the Immunomodulatory Properties of Three Probiotic Strains of *Lactobacilli* Using Complex Culture Systems: Prediction for In Vivo Efficacy

Erika Mileti, Gianluca Matteoli, Iliyan D. Iliev, Maria Rescigno*

Department of Experimental Oncology, European Institute of Oncology, Milan, Italy

Abstract

Background: While the use of probiotics to treat or prevent inflammatory bowel disease (IBD) has been proposed, to this point the clinical benefits have been limited. In this report we analyzed the immunological activity of three strains of *Lactobacillus* to predict their in vivo efficacy in protecting against experimental colitis.

Methodology/Principal Findings: We compared the immunological properties of *Lactobacillus plantarum* NCIMB8826, L. *rhamnosus* GG (*LGG*), *L. paracasei* B21060 and pathogenic *Salmonella typhimurium* (SL1344). We studied the stimulatory effects of these different strains upon dendritic cells (DCs) either directly by co-culture or indirectly via conditioning of an epithelial intermediary. Furthermore, we characterized the effects of these strains in vivo using a Dextran sulphate sodium (DSS) model of colitis. We found that the three strains exhibited different abilities to induce inflammatory cytokine production by DCs with *L. plantarum* being the most effective followed by *LGG* and *L. paracasei*. *L. paracasei* minimally induced the release of cytokines, while it also inhibited the potential of DCs to both produce inflammatory cytokines (IL-12 and TNF-α) and to drive Th1 T cells in response to *Salmonella*. This effect on DCs was found under both direct and indirect stimulatory conditions – i.e. mediated by epithelial cells - and was dependent upon an as yet unidentified soluble mediator. When tested in vivo, *L. plantarum* and *LGG* exacerbated the development of DSS-induced colitis and caused the death of treated mice, while, conversely *L. paracasei* was protective.

Conclusions: We describe a new property of probiotics to either directly or indirectly inhibit DC activation by inflammatory bacteria. Moreover, some immunostimulatory probiotics not only failed to protect against colitis, they actually amplified the disease progression. In conclusion, caution must be exercised when choosing a probiotic strain to treat IBD.

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Le sostanze secrete immunostimolanti

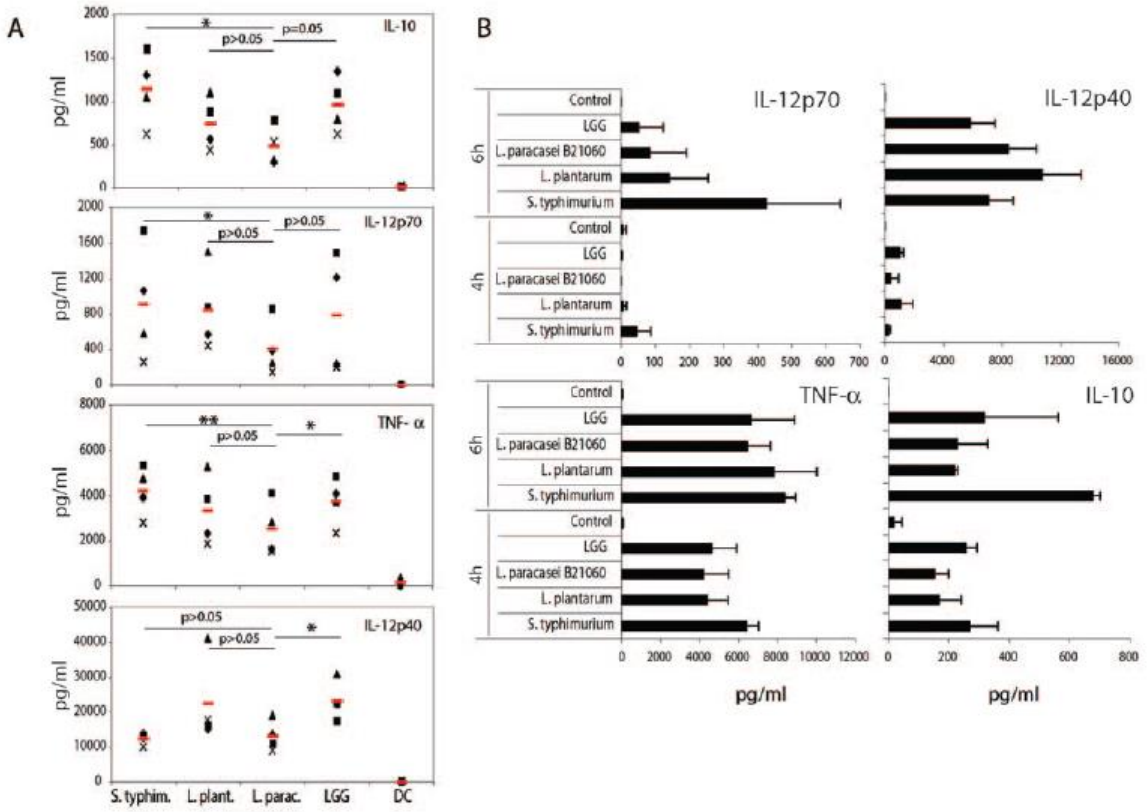

Figure 2. DCs incubated with different bacterial strains produce a distinct cytokine profile. A. DCs were incubated or not with the reported live bacterial strains for 1 h in medium without antibiotics, washed and incubated for 23 h in medium with antibiotics. Culture supernatants were collected and tested for cytokine contents by ELISA. Each symbol represents a different DC donor. Red lines represent mean values. *, $p<0.05$; **, $p<0.01$. S. typhim.: *S. typhimurium*; L. plant.: L. plantarum; L. parac.: *L. paracasei*. B. To analyze the kinetic of cytokine production, DCs were incubated or not with the reported live bacterial strains for 1 h in medium without antibiotics, washed and incubated for 3–5 h in medium with antibiotics. Culture supernatants were collected and tested for cytokine release by ELISA. Error bars: standard deviations on values obtained on 4 different donors.

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Le sostanze secrete immunostimolanti

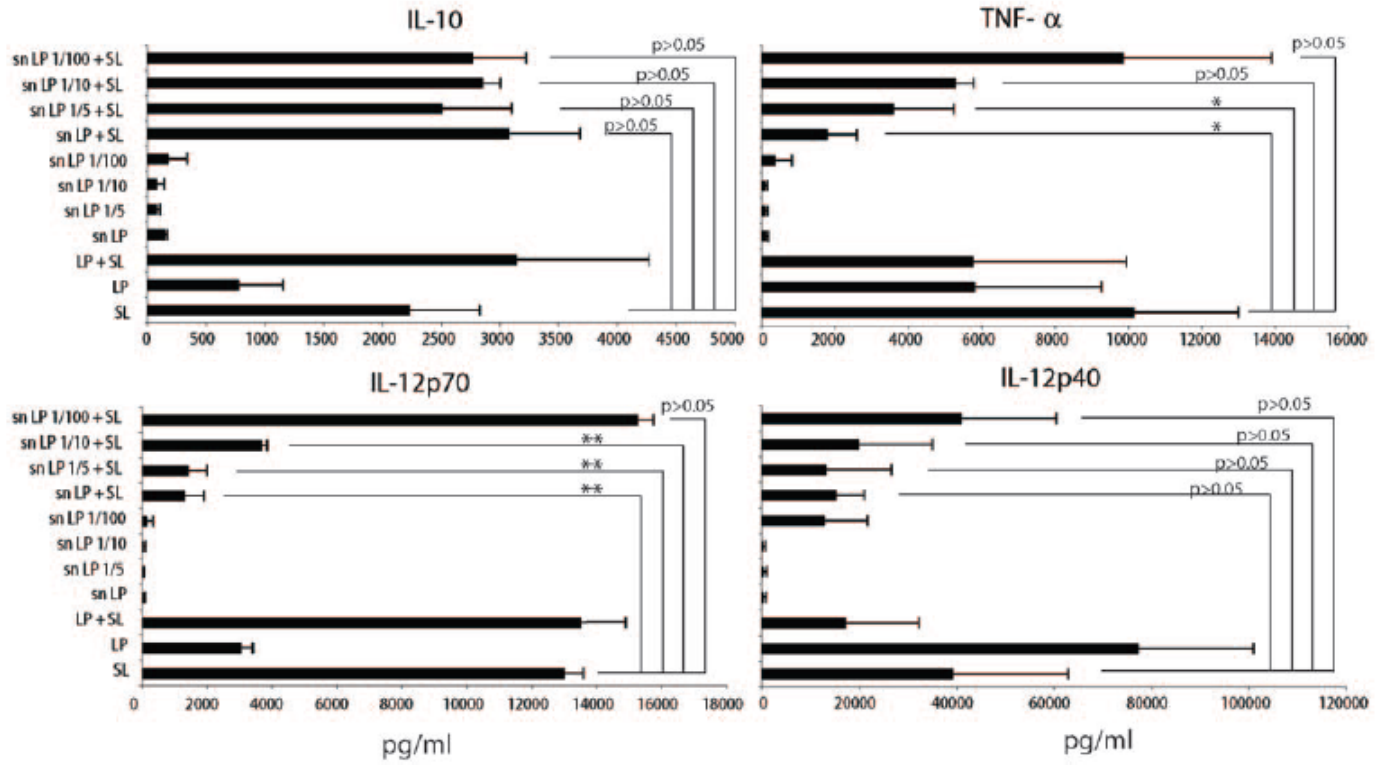

Figure 6. *L. paracasei* culture supernatant is responsible for the anti-inflamamtory activity of the bacterium. DCs were incubated or not with the reported live bacterial strains either separately (SL, *Salmonella*; LP, *L. paracasei*) or together (LP+SL) or in the presence of culture supernatants of *L. paracasei* corresponding to the exponential growth of the same amount of CFU of bacteria used to treat the DCs. The culture supernatant (sn LP) was used either undiluted or diluted 1/5, 1/10, 1/100 that correspond to nearly 7%, 1,4%, 0,7%, and 0,07% volume/volume of tissue culture medium, respectively. Cells were incubated with the different treatments for 1 h in medium without antibiotics, washed and incubated for 23 h in medium with antibiotics. Cytokine release was analyzed by ELISA. Error bars: standard deviations on values obtained on 3 different donors. *, $p<0.05$; **, $p<0.01$
doi:10.1371/journal.pone.0007056.g006

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Conclusioni

Le cellule batteriche hanno proprietà immuno-modulanti legate a strutture di superficie e/o sostanze secrete.
Queste proprietà sono ceppo specifiche.