Probiotics and systemic immunity

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Danone Research & BIOASTER

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Definition of probiotics

FAO & WHO definition:

“Probiotics are live microorganisms that, when administered in adequate amount, confer a health benefit to the host”
Beneficial health effects
Defense against pathogens

Local effect
- Gastrointestinal tract infections
- Antibiotic-Associated Diarrhea
- Travellers Diarrhea
- Etc...

Systemic and distal effect
- Parenteral vaccination
- Respiratory tract infections
- Urogenital infections
- Etc...

Immune modulation at systemic and distal mucosa
Probiotics and responses to vaccine
Probiotics & vaccine responses in seniors

A probiotic fermented dairy drink improves antibody response to influenza vaccination in the elderly in two randomised controlled trials

Thierry Boge, Michel Rémigy, Sarah Vaudaine, Jérôme Tanguy, Raphaëlle Bourdet-Sicard, Sylvie van der Werf

86 elderly in nursing home
Vaccination 2005-2006

N=44  L. casei CNCM I-1518 (Actimel®) 10^{10} cfu/2xday
N=42  Non fermented dairy control

Antibody titers measured by Haemagglutination inhibition test (HI test)

Vaccine. 2009; 27:5677-84.
Probiotics & vaccine responses in seniors

222 elderly in nursing home
Vaccination 2006-2007, 13 weeks product consumption


Antibody levels for B strain (GMT)

Seroconversion rate for B strain

- Significant higher antibody levels and higher seroconversion rate against the B strain over the time (3, 6 and 9 weeks post vaccination) in subjects consuming Actimel® as compared to control group.

- H1N1 and H3N2 were not significantly different between the 2 groups.
Probiotics and response to vaccine
Selection of clinical trials

**Study criteria:**
- Probiotic product with no concomitant treatment (unless identical in test and control group)
- Oral intake, concomitant with vaccination
- Parenteral vaccine
- Adults population
- Randomised, double-blind, placebo-controlled trial
- Publication in peer-reviewed journal, in English.

### Seniors
6 trials on influenza vaccine

### Young adults:
4 trials on influenza vaccine

#### 3 trials on influenza vaccine

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Vaccine</th>
<th>N</th>
<th>Method</th>
<th>Intervention effect</th>
<th>Trial reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. paracasei ssp paracasei Shirota fermented milk 6. 109 cfu twice daily</td>
<td>Parenteral influenza vaccine - campaign 2007-2008</td>
<td>565</td>
<td>HI</td>
<td>No significant difference on non-seroprotected sub-group (737-172 = 565) for H3N2.</td>
<td>van Puyenbroeck et al., Am J Clin Nutr 2012</td>
</tr>
</tbody>
</table>
**Probiotics and response to vaccine clinical trials in young adults**

### Young adults: 4 trials on influenza vaccine

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Vaccine</th>
<th>N</th>
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<tr>
<td>L. fermentum CECT5716 1010 cfu/day</td>
<td>Parenteral influenza vaccine - campaign 2004/2005</td>
<td>50</td>
<td>ELISA</td>
<td>Probiotic increased vaccine-specific IgA (no effect on IgG nor IgM) P&lt; 0,05</td>
<td>Olivares et al., Nutrition 2007</td>
</tr>
<tr>
<td>L. fermentum VR1003 109 cfu/ day</td>
<td>Parenteral influenza vaccine - campaign 2006</td>
<td>47</td>
<td>HI</td>
<td>Significant difference of H1N1 titers in Probiotic vs Placebo group. Non Seroconverted subject for H1N1 was lower in probiotic group (5,5%) vs 28% in placebo group.No difference for H3N2 nor B but very low response to vaccine for these 2 strains;</td>
<td>French &amp; Penny, Int J Probiotics and Prebiotics 2009</td>
</tr>
<tr>
<td>B. animalis ssp lactis BB-12 or L. paracasei ssp paracasei 431 1 x 109 cfu/day</td>
<td>Parenteral influenza vaccine - campaign 2008-2009</td>
<td>211</td>
<td>ELISA</td>
<td>Significantly greater increase in vaccine-specific plasma IgG titer in probiotic groups vs placebo, IgG1 and IgG3 (p&lt; 0,001)</td>
<td>Rizzardini et al. Br J Nut 2012</td>
</tr>
<tr>
<td>L. rhamnosus GG 1010 cfu twice daily</td>
<td>Nasal Live attenuated trivalent influenza vaccine - campaign 2007/2008</td>
<td>39</td>
<td>HI</td>
<td>Significant increase in seroprotection for H3N2 in probiotic group vs placebo (84% vs 55% p = 0,048). No effect on H1N1 nor on B strain, but very low vaccine response</td>
<td>Davidson et al., Eur j Clin Nutr 2011</td>
</tr>
</tbody>
</table>
Probiotics and resistance against respiratory infections
Probiotics & respiratory infections in senior

RCT in 1072 free-living elderly
76.0 years (median)

L. casei CNCM I-1518 (Actimel®) 10^{10} cfu/2xday
Non fermented dairy control
3 months during winter

Lower average and cumulated duration of URTI and rhinopharyngitis in probiotic group versus control

<table>
<thead>
<tr>
<th></th>
<th>Actimel®</th>
<th>Control</th>
<th>P-values^1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All CIDs</strong></td>
<td>N=104</td>
<td>N=111</td>
<td></td>
</tr>
<tr>
<td>Mean (SD), days</td>
<td>7.4 (5.6)</td>
<td>9.8 (7.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>Median. days</td>
<td>6.5</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td><strong>URTIs</strong></td>
<td>N=61</td>
<td>N=66</td>
<td></td>
</tr>
<tr>
<td>Mean (SD), days</td>
<td>7.7 (7.2)</td>
<td>11.0 (7.7)</td>
<td>0.00002</td>
</tr>
<tr>
<td>Median. days</td>
<td>7.0</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td><strong>Rhinopharyngitis</strong></td>
<td>N=61</td>
<td>N=58</td>
<td></td>
</tr>
<tr>
<td>Mean (SD), days</td>
<td>7.7 (7.2)</td>
<td>11.0 (8.1)</td>
<td>0.00007</td>
</tr>
<tr>
<td>Median. days</td>
<td>7.0</td>
<td>8.0</td>
<td></td>
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</table>

^1 Mann-Whitney

Evidence for an potential effect on RTI: lower number and occurrence of CID (87% RTI) in Actimel group versus control

RCT in 1000 healthy shift workers, 32.1 ± 8.9 years (mean ± SD)

L. casei CNCM I-1518 (Actimel®) 10^{10} cfu/2xday

Non fermented dairy control

3 months during winter

Subjects presenting CID (Number and %)

<table>
<thead>
<tr>
<th></th>
<th>Actimel</th>
<th>Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volunteers with infection, %</td>
<td>213 (43%)</td>
<td>256 (51%)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Proportion of CID during whole study

- URTI 59%
- LRTI 29%
- GITI 13%

Probiotics & respiratory infections
Selection of clinical trials

**Study criteria:**
- Probiotic product with no concomitant treatment (unless identical in test and control group)
- Oral intake
- Randomised, double-blind, placebo-controlled trials (parallel groups or cross-over)
- Results available on probiotics effect specifically on respiratory infection.
- Publication in peer-reviewed journal, in English.

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2001
Hatakka K et al., BMJ 322:1327

2005
de Vreese M et al., Clin Nutr 24:4
Weizman et al. Pediatrics.;115(1

2007
Hatakka K et al., Clin NutrJun;26
Marseglia GL et al. Ther Clin Risk
Tiollier et al., Mil Med.;172(9):1

2008
Cox AJ et al., Br J Sports Med, 2

2009
Leyer GJ et al., Pediatrics 124:e1
Rautava S et al., Br J Nutr 101:1;

2010
Hojsak I. et al., Clin Nutr. Jun;29

2011

2012
Cox AJ et al., Br J Sports Med, 2

2013
Nagalingam et al., Trends in Micro 2013

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*N* = 20

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**Figure 2:** Increase in published probiotic trials for treatment of respiratory diseases. Data generated by a PubMed search using the search-string ‘Probiotics + respiratory disease’. No studies were found before 1999 that matched the search criteria.
Young, middle aged Adults:
*De Vrese et al., 2005*

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Effect of probiotic bacteria (5 x 10^7 cfu/day) on symptoms of the common cold episodes with more than one symptom in detail during the observation period.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Probiotics+vitamins and minerals</td>
</tr>
<tr>
<td>Duration (days)</td>
<td>7.0 ± 0.5</td>
</tr>
<tr>
<td>Symptom scores (points)</td>
<td></td>
</tr>
</tbody>
</table>

**Meta-analyses on probiotics and RTIs**

Probiotics for preventing acute upper respiratory tract infections (*Hao et al., Cochrane Datab. Syst Rev. 2011 Sep 7;9*).

<table>
<thead>
<tr>
<th>Outcome or subgroup title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Number of participants who experienced URTI episodes: at least 1 event</td>
<td>6</td>
<td>1836</td>
<td>Odds Ratio (IV, Random, 95% CI)</td>
<td>0.58 [0.36, 0.92]</td>
</tr>
</tbody>
</table>

**Heterogeneity:** \( \text{Tau}^2 = 0.23; \text{Chi}^2 = 16.15, \text{df} = 5 (P = 0.01); I^2 = 69\% \)

Probiotics were better than placebo on acute URTI for the following criteria: number of participants experiencing at least one or three episode, rate ratio of URTI, antibiotic prescription. Probiotics and placebo were similar for the mean duration (MD) of an episode of acute URTI.
Mechanisms of action
Impact on systemic immunity

Probiotic

Systemic & distal effect

- Parenteral vaccination
- Respiratory tract infections

Clinical endpoints

Immunological markers

- Blood

Gut Immune system (IS)

Systemic IS

Distant mucosal IS

Unknown connections
In case of infections, Leukocytes and Neutrophils blood counts were higher in Actimel group versus control. No difference in non-infected subjects.

In case of infection, NK cells blood counts were higher in Actimel group versus control. No difference in non-infected subjects.

Effect of probiotics on Dendritic cells in absence of infectious signal

In absence of inflammatory signal, induction of hyporesponsive suppressor T cells by probiotics-treated DCs, that will reinforce the gut tolerance

Baba et al., J Leukocyte Biol 2008
Effect of *L. casei* CNCM I-1518 on Dendritic cells in presence of infectious signal

Interleukin (IL)-12p70 profiles of DCs stimulated with *Lactobacillus casei* CNCM I-1518 + various TLR agonists

DCs stimulated with *L. casei* plus poly(I:C) induces a higher amounts of IFN-γ in CD4+ T cells co-culture

In presence of an infectious signal (poly (I:C)), conversion of *L. casei* -treated DCs into potent promotors of Th1 cells.

**Note**: IL-12 stimulated production = unique profil of *L. casei* among eight different bacterial strains tested

*Baba et al. (2009) Immunology 128, e523*
Effect of probiotic on immunity in presence of infectious signal

**In vitro**

- **probiotic**
- Immature DC
- Maturation
- Mature DC
- + poly(I:C)
- IL-12
- $T_{H1}$
- $T_{H17}$
- $T_{Reg}$
- $T_{H2}$

**In vivo**

- Oral administration of probiotic ($L$ rhamnosus Lr1505 or Lr1506) 5 days
- Then, mice intranasaly infected with poly(I:C)

**Results**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lr1505</th>
<th>Lr1506</th>
</tr>
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<tbody>
<tr>
<td>IFN-γ</td>
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</table>

**Lung (BAL)**

- $CD3^+CD4^+IFN-γ^+$

Villena et al., BMC Immunol 2012, 13:53
Probiotics have been shown in clinical trials to confer health benefit beyond the gut area:
- Increase antibody response to parenteral vaccine
- Increase resistance to respiratory tract infections (incidence, duration and/or severity)

Mechanisms by which orally administered probiotics impact systemic and distal mucosal immunity need to be further dissected both at
  • At the host level
  • At the probiotic level
Thanks to

<table>
<thead>
<tr>
<th>Vaccination studies</th>
<th>RTI studies</th>
<th>DCs in vitro studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thierry Boge</td>
<td>F Lacoin</td>
<td>Nobu Baba</td>
</tr>
<tr>
<td>Michel Remigy</td>
<td>S de La Motte</td>
<td>Manuel Rubio</td>
</tr>
<tr>
<td>Sylvie van der Werf</td>
<td>J. Schrezenmeir</td>
<td>Marika Sarfati</td>
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<tr>
<td>Sarah Vaudaine</td>
<td>Eric Guillemard</td>
<td>Sandrine Samson</td>
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<td>Sandrine Samson</td>
<td>A.L Flavigny</td>
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<tr>
<td>Jérome Tanguy</td>
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<td>Francoise Tondu</td>
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