Probiotics in Pediatrics

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Normal Flora

- Over 400 species, more bugs than cells
- Most acquired perinatally
- Maternal vaginal and fecal flora
- Some environmental acquisition early in life
- May differ in caesarian section infants
Why Bother with Gut Microflora?

- Important in immune regulation
- Important in gut barrier function
- Important in nutrient metabolism in absorption
- Potential source of infection
There are Two Ways to Modify Gut Flora

• **Probiotics**
  
  = Human derived (usually) exogenous strains of bacteria that may be provided by diet or supplement to provide a specific health benefit

• **Prebiotics**
  
  = Poorly digested oligosaccharides that promote the growth of desirable stains of commensal bacteria

• They are not as similar as their names implies
What Prebiotics Do

• Increase number of less aggressive bacteria in the bowel
• Produce short chain fatty acids in the bowel which strengthen the lining and protect against invasion
• Lower the intestinal pH which might enhance calcium absorption
• Normally present in breast milk, enhance certain strains already present
• Immunomodulation?
What Probiotics Do

• Redirect the immune system toward fighting infections and away from allergy
• Block access of pathogens to receptors?
• Stimulate other parts of the intestinal defense system (i.e. mucin)
• Remember, these are foreign bacteria not recognized by the immune system and therefore, they elicit an immune response
Common Probiotics

• Bacteria: several strains of
  – *Lactobacillus*
  – *Bifidobacterium*

• Yeast
  – *Saccharomyces boulardii*

• Killed organisms and subfractions
Important Concepts

- Benefits of probiotics are strain-specific
- These must be proven for each strain in randomized clinical studies
- Not all proven strains are appropriate for all indications
- Think of probiotics like antibiotics, pick the strain proven for the specific disorder you want to prevent or treat
Canis Familiaris
How to Evaluate a Probiotic

- Shelf survival (Is it dead?)
- Colonization (Is it found in the stool?)
- Clinical studies (Are they positive and relevant to the intended goal?)
- Do you have the strain you want?
Persistence of *Lactobacillus GG* After Discontinuing Feeding

- **No. of GG (log_{10}) mL/feces**
- **Positive for GG (%)**

Days after stopping GG

* = Mean  SE  
**% = No. of positives/no. of subjects x 100

Probiotics in Acute Diarrhea: What Has Been Proven?

Some Probiotics:

- Reduce duration, severity of viral diarrhea
- Reduce risk of traveler’s diarrhea
- Reduce incidence of diarrhea in day care centers
- Reduce relapsing *Clostridium difficile* diarrhea
- Reduce incidence of antibiotic-associated diarrhea
Duration of Diarrhea


*p≤0.001 vs placebo

LGG Milk | LGG Powder | Placebo
---|---|---

Number of days

* * *
Duration of Diarrhea

Number of days

LGG (n = 16)

Lactophilus (n = 14)

Yalacta (n = 19)
LGG in Acute Diarrhea

Guandalini S et al. JPGN 2000;30:54-60

Duration of diarrhea (days)

Placebo

LGG

- All (n=287)
- Rotavirus + (n=101)
- Rotavirus - (n=186)

p<0.05 LGG vs placebo
Lactobacillus reuteri in Acute Diarrhea in Children

- Frequency of watery stools (no. per day)
- Days after therapy initiation

Shornikova A-V et al. JPGN 1997:399-404
Limitations of Probiotic Therapy in Acute Severe Diarrhea

- 124 infants with three or more watery stools per day
- *Lactobacillus GG* or placebo initiated at the time of oral electrolyte therapy
- Duration of diarrhea 38 hours treatment group versus 39 hours placebo group (NS)
- Stool output 140 (treatment) versus 184 ml per kg per day (placebo) (NS)
Duration of Diarrhea Expressed in the Form of Kaplan-Meier Survival Estimates (P>0.1)

Systematic Review of Published Randomized, Double-blind, Placebo-controlled Trials (Meta-analysis)

- Modest but clinically significant benefit in treatment of acute gastroenteritis, especially with *Lactobacillus GG* (10 studies)
- Further research needed to establish efficacy of other strains (strains lumped together for analysis)
- Cannot draw firm conclusions regarding prevention because of heterogeneity of studies

Szajewska H & Mrukowicz JZ. JPGN 2001;33:S17-S25
Prevention of Acute Diarrhea in a Brazilian Daycare Center

- Large public facility
- Cares for 200 children each day (6 weeks to 5 years of age)
- Children eat all meals at daycare
- Serves economically disadvantaged
- Weekend meals sent home

Costa-Ribeiro H et al. JPGN 2000;31:S252
Distribution of Diarrhea in Treatment and Placebo Groups in Daycare Centers

<table>
<thead>
<tr>
<th></th>
<th>LGG</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Month</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>2 Months</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

N = 119

Costa-Ribeiro H et al. JPGN 2000;31:S252
LGG in Daycare Center in Finland

- 18 day care centers; 571 healthy children participated (282,289)
- Milk with or without LGG
- Antibiotics for respiratory infections reduced (P=0.03)
- Reduction modest

Hatakka K et al. BMJ 2001;322:1327-9
L. reuteri vs. BB12: Prevention of Daycare Illness

Treatment Effect of LGG on Respiratory Tract and GI Infections in Children Attending Daycare

Abdović S et al. ESPGHAN 2009; 59:PN1-02.

- Respiratory Infection
- GI Infections

Placebo (n=142) vs LGG (n=139)

*p<0.001
**p=0.079
# Nosocomial Respiratory Tract and GI Infections in Children Receiving LGG


<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=366)</th>
<th>LGG (n=376)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Respiratory Infection</strong></td>
<td><strong>Placebo (n=366)</strong></td>
<td><strong>LGG (n=376)</strong></td>
</tr>
<tr>
<td>Number of Children</td>
<td>15</td>
<td>20</td>
</tr>
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</table>

**p=0.017**

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=366)</th>
<th>LGG (n=376)</th>
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</thead>
<tbody>
<tr>
<td><strong>GI Infections</strong></td>
<td><strong>Placebo (n=366)</strong></td>
<td><strong>LGG (n=376)</strong></td>
</tr>
<tr>
<td>Number of Children</td>
<td>25</td>
<td>45</td>
</tr>
</tbody>
</table>

**p<0.001**
Prevention of Diarrhea in Hospitalized Infants

*Bifidobacterium lactis* (BB12)  
*Streptococcus thermophilus*

- Double-blind, placebo-controlled trial
- 55 infants age 5–24 months
- 4447 patient days
- Diarrhea in 8/26 with control formula
- Diarrhea in 2/29 with supplemented formula

Incidence of Diarrhea (Main Outcome)

Incidence (%)

LGG | Placebo
---|---
n=45 | n=31
Lactobacillus GG for Travelers’ Diarrhea in Developing Countries

Protection rate = 47% (P=0.05)

Hilton E et al, J Travel Med 1997;4:41-3
LGG Research: Travelers’ Diarrhea

Probability of remaining well

Time (days)

Placebo

Lactobacillus GG

Antibiotic-associated Diarrhea: Organisms Studied

- *Saccharomyces boulardii*
- *Lactobacillus*
  - several studies
- *Bifidobacterium*
Antibiotic-associated Diarrhea: Pediatric Prevention Study

- *Lactobacillus GG*
- 200 children
  - (100 probiotic, 100 placebo)

Antibiotic-associated Diarrhea Prevention

*Saccharomyces boulardii*

- Adam et al. 1977 (n=388)
  - 4.5% treatment vs 17% control
- Surawicz et al. 1989 (n=180)
  - 9.5% treatment vs 22% placebo
LGG study: Incidence of Diarrhea (Day 9)

Stool Consistency Scores of ≤4

Antibiotic-associated Diarrhea in Pediatrics

- Study in 119 patients (mean age 4.5 yrs)
- Children receiving wide variety of antibiotics
- Incidence of diarrhea
  - 5 % LGG group vs 16% placebo (P=0.05)

Arvola T et al. Pediatrics 1999;104:e64
Probiotics in the Prevention of Antibiotic-associated Diarrhea

**Meta-analysis**

- 9 randomized, double-blind, placebo-controlled trials
- 2 in children, 4 yeast, 4 *Lactobacilli*, 3 with combinations of bacteria
- Odds ratio in favor of active treatment was 0.39, $P<0.001$ for yeast and 0.34, $P<0.01$ for *Lactobacilli*

D'Souza AL et al. BMJ 2002;324:1361
Mechanisms of Protection: Diarrheal Disease

- Competitive exclusion
- Stimulation of intestinal mucin secretion
- Stimulation of immune response
Serum IgA Antibody Response to Rotavirus in Convalescent Stage

Effect of LGG on Antibody Response to Typhoid Vaccine

Expression Levels of *MUC2* & *MUC3* mRNA from HT29 Cells

Mack DR et al. Gut 2003;52:827-33
Lactobacillus casei Abundance Is Associated with Profound Shifts in the Infant Gut Microbiome

Michael J. Cox, Yvonne J. Huang, Kei E. Fujimura, Jane T. Liu, Michelle McKean, Homer A. Boushey, Mark R. Segal, Eoin L. Brodie, Michael D. Cabana, Susan V. Lynch

Figure 2. Hierarchical cluster analysis of infant stool samples. Hierarchical cluster analysis reveals that LGG abundance is associated with specific bacterial community structures. doi:10.1371/journal.pone.0008745.g002
New Areas with Interesting Data

- Inflammation
- Allergy
- Obesity?
Probiotic Cocktail in NEC

Table III. NEC and mortality

<table>
<thead>
<tr>
<th></th>
<th>Study group (n = 72)</th>
<th>Control group (n = 73)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td># cases NEC</td>
<td>3 (4%)</td>
<td>12 (16.4%)</td>
<td>P = .03*</td>
</tr>
<tr>
<td>BW of NEC infants (g)</td>
<td>949 ± 223</td>
<td>956 ± 223</td>
<td>P = .85</td>
</tr>
<tr>
<td>GA of NEC infants</td>
<td>26.8 ± 1.6</td>
<td>27.6 ± 1.9</td>
<td>P = .52</td>
</tr>
<tr>
<td>Apgar 1 min</td>
<td>4 ± 1</td>
<td>6 ± 2</td>
<td>P = .08</td>
</tr>
<tr>
<td>Apgar 5 min</td>
<td>7 ± 2</td>
<td>8 ± 1</td>
<td>P = .58</td>
</tr>
<tr>
<td>Age of diagnosis (d)</td>
<td>21 ± 9</td>
<td>21 ± 14</td>
<td>P = 1.00</td>
</tr>
<tr>
<td>Bell staging</td>
<td>1.33 ± 0.46</td>
<td>2.33 ± 0.46</td>
<td>P = .005*</td>
</tr>
<tr>
<td>NEC-associated mortality</td>
<td>0/3</td>
<td>3/12</td>
<td>P = .87</td>
</tr>
<tr>
<td>NEC and/or death</td>
<td>6/73</td>
<td>17/72</td>
<td>P = .025*</td>
</tr>
</tbody>
</table>

Necrotizing Enterocolitis (NEC)

- *L. acidophilus* vs. *B. infantis*
- 367 preterm infants
- Death: 7 vs. 20, $P=0.009$
- Death or NEC: 9 vs. 24, $P=0.009$

Meta-analysis in NEC of 9 clinical trials

- Probiotics Reduce the Risk of Necrotizing Enterocolitis in Preterm Infants: A Meta-Analysis.
- **Alfaleh K, Anabrees J, Bassler D.**
- Division of Neonatology, Department of Pediatrics, King Khalid University Hospital and College of Medicine, King Saud University, Riyadh, Saudi Arabia.

Probiotics in Crohn’s Disease

• VSL#3 prevents recurrence of pouchitis and may be useful in ulcerative colitis, **BUT** no benefits shown to date in Crohn’s disease

• *E. coli* strain Nissle may be effective in UC

• LGG studies have been disappointing
Randomized, Double-blind Trial of *Lactobacillus GG* versus Placebo in Crohn’s Disease

- 75 children aged 5–21 years with Crohn’s disease in remission
- Randomized to LGG or placebo and followed for up to 2 years; time to relapse measured
- Median time to relapse was 9.8 months in LGG group and 11 months in placebo group
- 31% of patients in LGG group relapsed vs. 17% in placebo group
- Differences were not statistically significant and no benefit of probiotics was apparent

Bousvaros A et al. Inflamm Bowel Dis 2005;11:833-9
Adult Irritable Bowel Syndrome (IBS)

- *L. salivaricus* vs. *B. infantis* 35624
- 77 adult IBS patients randomized
- *B. infantis* reduced pain, bloating, and difficulty with defecation vs. *L. salivaricus* and placebo
- PBMC IL10/IL12 ratio increased with *B. infantis*
- IBS may be an inflammatory disease and may be treated with this probiotic strain
- No studies yet in pediatric IBS

O'Mahony L et al. Gastroenterology 2005;128:541-51
Other Inflammatory Conditions: Allergy

- LGG is beneficial in atopic dermatitis and allergic colitis
- Some other strains may also work but more data are needed
- Benefits are likely to be limited to infants and small children, and do not include asthma yet
Extent of Rash in Infants with Milk Allergy

Lactobacillus GG in Allergic Infants: Additional Results

- Eosinophil cationic protein - trend toward reduction after one month of treatment
- Alpha-1 antitrypsin level in stool was significantly decreased
- Fecal tumor necrosis factor decreased significantly

Majamaa H & Isolauri E. J Allergy Clin Immunol 1997;99:179-85
“Improved Recovery From Cow Milk Allergy Colitis with *Lactobacillus GG* Compared to Extensively Hydrolyzed Formula Alone”

- 26 formula-fed infants with hematochezia
- All were diagnosed with cow milk allergy colitis
- 4 week period of dietary antigen elimination
- Randomly assigned to formula feeding
  - Extensively hydrolyzed formula
  - Extensively hydrolyzed formula with LGG
- Formulas were fed for 1 month
- Measures of effectiveness:
  - Fecal calprotectin (FC) used as a surrogate marker of digestive distress
  - Occult blood in stool

Occult blood stool after 4 wk. of dietary treatment

<table>
<thead>
<tr>
<th></th>
<th>Nutramigen LGG</th>
<th>Nutramigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

\[ X^2 = 11.798 \quad p=0.001 \]
Mean Decrease (and 95% CI) in Fecal Calprotectin in Colitis (group A) and Controls (Group B) after 4 wk.

<table>
<thead>
<tr>
<th>Type of milk</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutra LGG</td>
<td>-214 (-283-146)</td>
<td></td>
</tr>
<tr>
<td>Nutra</td>
<td>-113 (-173-52)</td>
<td></td>
</tr>
<tr>
<td>Formula</td>
<td></td>
<td>-37 (-59-16)</td>
</tr>
</tbody>
</table>

p<0.0001
Cows Milk Protein Tolerance at Months
Berni Canani, ESPGHAN 2010
Probiotics Prevent Atopic Disease

• Double-blind, randomized, placebo-controlled trial
• *Lactobacillus* GG given to pregnant women with a first-degree relative with atopic eczema
• Newborn infants continued to receive LGG
• Frequency and severity of atopic eczema were evaluated in children at age 2

Treatment Effect of *Lactobacillus* GG in 2-yr old Children with Atopic Eczema
Atopic Dermatitis: 4 Strains Together

• DBPC trial of LGG, mixture of probiotics, placebo
• IgE positive group responded, but only to LGG and not to mixture (probiotic cocktails should be viewed with great skepticism)
• Non IgE positive group did not respond

Probiotic Safety

• Woman with diabetes developed a liver abscess while consuming LGG drink
  – A strain of *Lactobacillus* indistinguishable from LGG was cultured from the abscess
  – treated without complications

• Sepsis reported in some children with indwelling CV lines who received LGG capsules that had been broken open

Saccharomyces boulardii: Disseminated Infections

- Several cases of fungicemia
  - many on multiple antibiotics
- All were being treated with oral Saccharomyces
- Central lines were usually present
- All responded to fungicidal therapy

## Lactobacillus GG (CFU/g)

<table>
<thead>
<tr>
<th></th>
<th>Time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>GG #1</td>
<td>3.7 (10^{10})</td>
</tr>
<tr>
<td>GG #2</td>
<td>3.3 (10^{5})</td>
</tr>
<tr>
<td>GG #3</td>
<td>8.3 (10^{4})</td>
</tr>
<tr>
<td>GG #4</td>
<td>+</td>
</tr>
<tr>
<td>GG #5</td>
<td></td>
</tr>
<tr>
<td>Cont #1</td>
<td>0</td>
</tr>
<tr>
<td>Cont #2</td>
<td>0</td>
</tr>
<tr>
<td>Cont #3</td>
<td>0</td>
</tr>
</tbody>
</table>

Perinatal and Neonatal Manipulation of the Intestinal Microbiome: a Note of Caution

• Considerable enthusiasm for the routine use of probiotics in neonates has emerged, despite a lack of knowledge about long-term effects.

• We need to be cognizant of long-term effects.

• Previous studies suggest that inactivated probiotic bacteria, their DNA, and/or soluble products are likely to provide the beneficial properties of live bacteria, but may be safer alternatives because the dose of these agents can be readily controlled and they are less likely to establish lifelong niches that may be difficult to undo.

Josef Neu, MD
Summary: Probiotics

• Certain probiotics are useful in a variety of diarrheal diseases
• Some probiotics may be useful in inflammatory and allergic disorders
• Affects are strain specific
• Clinical studies needed with each strain and disorder
• Probiotics appear to be safe, even for infants, unless they have central lines
• Most positive data on *Lactobacillus* strains (not *acidophilus*), especially LGG and *L. reuteri*
Concluding Comments

- Probiotics should be considered much as we consider antibiotics
- We should select the probiotic that is effective and appropriate to the indication
- There are no data to suggest value in treating everyone with probiotics (we would not do this with antibiotics either)