Immunopharmacology of non-digestible carbohydrates: a breakthrough for clinical nutrition?

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Zurich
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60 - 70% of the current drugs have their origin in edible plants, fruits, vegetables, herbals or ferments.
"Let medicine be thy food, and food be thy medicine."

Hippocrates of Cos, Greece
460-377 B.C.
Overlap between Nutrition and Pharma
Health claims/Immune claims/Reimbursement

What do we need?

- Reliable and high quality science
- Regulation/Guidelines (FDA / medical food / EFSA / FSMP)
- Laws and inspection
Translational Research – WHO / ILSI / FDA / TIP

- In vivo Animal
- In vitro Animal Cells
- In vitro Human Cells
- In vitro Mechanistic

- In vivo Human
Bone marrow
Lymph nodes
Spleen
Thymus

Organs

60 – 70 % of immune cells in the gastro-intestinal tract !!

Cells

Epithelial cell
Granulocyte
Macrophage / Dendritic cell
Monocyte
T Lymphocyte
(Th1, Th2, Th3, Tr, ...)
B Lymphocyte / Antibodies
Natural Killer Cell
**Immune system**

- **Innate immune system**
  - 1st line of defense (Non-specific)
  - Macrophage
  - Natural Killer (NK) cell

- **Adaptive immune system**
  - 2nd line of defense (Specific)
  - T lymphocyte
  - B lymphocyte
Hyper immune-responsiveness:
- Allergy
- Autoimmunity
- Chronic inflammatory diseases

Hypo immune-responsiveness:
- Infections
- Tumors/metastasis
Immune regulation

Resistance to infections

Th1/Th17
Th0
Th2

Allergy

DCs

Th2-inducing innate stimuli? (Helminth or fungal product, Allergen)

Dendritic cells

Toll-like receptor

Microbial components (LPS, CpG DNA etc)

Innate immunity

Antigen presentation

Costimulatory molecules

IL-12, IL-18

Adaptive immunity

T_{n}1

IFN-{\gamma}

T_{h}2

IL-4

Naive T

?'
Immune regulation

Cellular immunity:
Th1: IL-2, IL-3, IL-12, IFN-γ, IL-7, IL-15, IL-23
Th2: IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13
Tr/Th3: IL-10, TGF-β1 (inhibitory cytokines)

Humoral Immunity:
Th1: IgG1, IgG3
Th2: IgG2, IgE
Tr/Th3: IgG4
Immune regulation in the intestine: a bridge to systemic immunity
Immune disorders

- HIV
- COPD
- Allergies
- Asthma
- Atopic eczema
- Coeliac disease
- Cystic Fibroses
- Cancer
- Elderly
- Infants

Th1 ↓, Th2 ↑, Th1/Th2 ↓

Th1 ↑

Th2 ↑ (type I allergy)

Th2 ↑

Th1 ↑

Th1 ↑?

Th1 ↓

Th1 ↓

Th1 ↓, Th2 ↑
Th1 and Th2 activity as a function of age

- Genes
- Hygiene
- Drugs
- Diets
- Stress
- Hormones

![Graph showing Th1 and Th2 activity as a function of age.](image)
Compounds with immunological properties in human milk

**Anti-microbial compounds**
- Immunoglobulins: sIgA, sIgG, sIgM
- Lactoferrin, lactoferrin B and H
- Lysozyme
- Lactoperoxidase
- Nucleotide-hydrolizing
- Antibodies
- \( \kappa \)-casein and \( \alpha \)-lactalbumin
- Haptocorrin
- Mucins
- Lactadherin
- Free secretory component
- Oligosaccharides and pre-biotics

**Immune development compounds**
- Macrophages
- Neutrophils
- Lymphocytes
- Cytokines
- Growth factors
- Hormones
- Milk peptides
- Long-chain polyunsaturated fatty acids
- Nucleotides
- Adhesion molecules

**Anti-inflammatory compounds**
- Cytokines: IL-10 and TGF\( \beta \)
- IL-1 receptor antagonist
- TNF\( \alpha \) and IL-6 receptors
- sCD14
- Adhesion molecules
- Long-chain polyunsaturated fatty acids
- Hormones and growth factors
- Anti-idiotypic antibodies
- Hormones and growth factors

Field J Nutr 2005
Translational research – WHO/ILSI/FDA/TIP

- **In vivo**
  - Animal
  - Human

- **In vitro**
  - Animal Cells
  - Human Cells

**In vitro Mechanistic**
scGOS e.g. DP3

\(\text{Gal(}\beta\text{1-4)Gal(}\beta\text{1-4)Glc}\)

IcFOS e.g. DP10

\([\text{Frc(}\beta\text{2-1})]_8\text{Frc(}\beta\text{2-1)Glc}\)

90 % GOS: short-chain \(\beta\)-Galacto-OligoSaccharides from lactose

10 % FOS: long-chain \(\beta\)-Fructo-OligoSaccharides from chicory
Inflammation induced tissue destruction

Intestinal Immune Cell activity

Epithelial Function and Regeneration

Permeability

Mesenchym

Mucus SCFA, SIgA

Improvement gut barrier? – First line defense
Oligosaccharides

SCFA differentially stimulate mucin production: mono and co-cultures

Linette Willemsen et al, Gut 2003
scGOS/lcFOS stimulates TLR9 induced IFN-γ production by human blood cells in co-culture systems with human gut epithelial cells

Kievit and Willemsen, 2009
IcFOS stimulates NO\textsubscript{2} production by murine MØ

Jeurink et al, unpublished observations
**Vaccination model**

**Study parameters:**
- DTH
- Influenza specific antibody titers
- Ex-vivo lymphocyte restimulation

**Timeline:**
- Start dietary intervention
- Blood sample
- Primary influenza immunization
- Blood sample
- Booster influenza immunization
- DTH
- End
GOS/IcFOS improves vaccination

Conclusions so far

- GOS/LcFOS can affect systemic immunity (Th1/Th2)
- Regular T cells play a crucial role
- There is synergy between different types of oligosaccharides
- Not all oligosaccharides affect the immune system

- What about improved resistance to infections
- What about reduced risk for IgE mediated allergy
Immune disorders

- HIV
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Th1 ↓, Th2 ↑, Th1/Th2 ↓

Th1 ↑

Th2 ↑ (type I allergy)

Th2 ↑

Th1 ↑

Th1 ↑?

Th1 ↓

Th1 ↓

Th1 ↓, Th2 ↑
Immune skewing

- Microbes → TLRs
- Oligosaccharides → C-type lectins, DC-sign

Diagram showing interaction between microbes, TLRs, and immune cells, leading to skewing of immune response towards TH1 or TH2 cytokines.
scGOS/IcFOS affects Th1/Th2 via DC/T-cell interaction

Stimulation of anti-allergic profile
Allergy (respiratory)

GOS/IcFOS impairs allergic inflammation in the lungs

Number of broncho alveolar cells (x 10⁴)

Non-sens | Sens | Sens+oligo's

P<0.001 | P<0.05

Allergy (respiratory)

GOS/IcFOS impairs IgE in serum

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Th1↓, Th2↑, Th1/Th2↓

Th1↑

Th2↑ (type I allergy)

Th2↑

Th1↑

Th1↑?

Th1↓

Th1↓

Th1↓, Th2↑
Cytomegalo virus infection model in mice
(systemic infection)

Systemic CMV

14 days

0-6 days

KRA-anesthesia
Urine / Blood / Section Organs

Suppletion oligosaccharides in AIN93-G chow
Oligosaccharides decrease MCMV load \textit{in vivo}

Infectious virus detection in C57BL/6J liver

- Control
- Oligo

Time postinfection (days)

Positive plaque assay (%)
Translational research – WHO/ILSI/FDA/TIP

- **In vivo**
  - Animal

- **In vitro**
  - Animal Cells
  - Human Cells
  - Mechanistic

- Infants / Children
Atopic Dermatitis in high-risk infants at 6 months

Moro et al., Arch. Dis. Child, 2006
GOS/IcFOS decrease serum IgE in high-risk infants at 6 months

E. Van Hoffen et al., Allergy 2009
Early dietary intervention with a mixture of prebiotic oligosaccharides reduce the incidence of infections during the first 2 years of life

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>scGOS/IcFOS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>episode/infant</td>
<td></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>68</td>
<td>66</td>
</tr>
<tr>
<td>Physician-diagnosed infections</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (any kind of infection)**</td>
<td>5.9 ± 4.1</td>
<td>4.1 ± 3.1</td>
</tr>
<tr>
<td>URTI†</td>
<td>3.2 ± 2.2</td>
<td>2.1 ± 1.8</td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>1.3 ± 0.8</td>
<td>0.9 ± 1.1</td>
</tr>
<tr>
<td>Otitis media</td>
<td>0.7 ± 1.2</td>
<td>0.5 ± 1.0</td>
</tr>
<tr>
<td>Gastrointestinal infections</td>
<td>0.6 ± 0.9</td>
<td>0.4 ± 0.7</td>
</tr>
<tr>
<td>Urinary tract infections</td>
<td>0.1 ± 0.5</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td><strong>Infections requiring antibiotic prescriptions</strong></td>
<td>2.7 ± 2.4</td>
<td>1.8 ± 2.3</td>
</tr>
<tr>
<td><strong>Fever episodes recorded by parents†</strong></td>
<td>3.9 ± 2.5</td>
<td>2.2 ± 1.9</td>
</tr>
</tbody>
</table>

† Values are means ± SD. *Different from placebo, $P < 0.05$, **$P = 0.01$, †$P < 0.01$, ‡$P < 0.0001$.

Arslanoglu et al., 2008, J. of Nutr. 138:1091-1095
Early dietary intervention with a mixture of prebiotic oligosaccharides reduce the incidence of acute diarrhoea and protect from recurrent URTI

| Table 2 Primary outcomes (intention to treat analysis) |
|----------------------------------------|-----------------|-----------------|-----------------|
| **Gastrointestinal infections**        | GOS/FOS group   | Formula Standard group | P value |
| Number of episodes of diarrhea         | 22              | 44              | .02             |
| Number of children with 1 episode of diarrhea | 19/169             | 37/173           | .0129           |
| **Upper respiratory tract infections** |                 |                  |                 |
| Number of episodes of URTI            | 241             | 302             | NS              |
| Number of children with at least 1 URTI | 84/169             | 87/173           | NS              |
| Number of children with > 3 URTI      | 22/169           | 36/173           | .06             |
| **Use of antibiotics for URTI**       |                 |                  |                 |
| Number of antibiotic courses/URTI     | 123/241         | 190/302         | NS              |
| Number of children with at least 1 antibiotic course | 65/84            | 78/87           | .038            |
| Number of children with ≥2 antibiotic courses | 32/84            | 59/87           | .0001           |
| Number of children with ≥3 antibiotic courses | 14/84            | 34/87           | .0012           |

*Bruzzesse et al., 2009, Clinical Nutrition, in press*
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- Th2 ↑
- Th1 ↑
- Th1 ↑?

- Th1 ↓
- Th1 ↓

Th1 ↓, Th2 ↑
Summary

From invited review on oligosaccharides: critical reviews immunology: Paul Vos et al., 2007
Conclusion (1)

- 60 - 70% of the immune cells are present in the gastro-intestinal tract
- There is a crucial interaction between gut associated and systemic immunity
- Non-digestible carbohydrates can affect the immune system both locally as well as systemically (less infections, less allergic inflammation) > a new chapter in immuno-pharmacology
Conclusion (2)

- Non-digestible carbohydrates are a promising tool to improve immune responsiveness in immuno-compromised individuals (cancer, HIV, elderly,..)

- Food immunology is one of the most promising new life sciences at the interface between food and pharma

- Translational bi-directional research is essential
“There is a reason behind everything in nature.”

Aristoteles