The evolving gut microbiome in early life:

Infant feeding and allergy outcomes

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And
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Allergic diseases;

- Are increasing in prevalence world-wide
- Pose a large health/economic burden
- Are significantly affected by early life environment
  - Nutrition
  - Allergen exposure
  - Microbial experience

Are controllable, and not curable, but may be preventable by environmental manipulation
Mechanisms

• The foetus exists in an allergy promoting environment which is a component of regulation of Th-1 mediated rejection responses to foeto-paternal antigens
• All neonates consequently have a Th-2 biased immune response
• Post-natal gut exposure to allergen in the presence of a “normal” microbiome and no co-stimulation regulates the Th-2 bias and induces tolerance
• Skin and airway exposure to allergen prior to tolerance induction increases the risk of sensitisation accentuated by the presence of genetic or acquired epithelial barrier defects
Allergy as a consequence of an epidermal/mucosal barrier defect

Intact epithelium

Epithelial barrier defect

Genetically or environmentally determined and enhanced by co-existent inflammation

Systemic food and inhalant sensitisation

Beck LA, Leung DY. JACI 2000
Appropriate exposure induces tolerance.

No co-stimulatory signals with exposure and normal microbiome results in non-responding T-lymphocytes (anergy), and deletion of any sensitised cells (clonal deletion).
Appropriate microbiome facilitates tolerance induction and T-cell regulation

**Microbiome**

- Competitive inhibition of pathogen adherence, and bacteriocidal activity, preventing co-stimulation

**Migration to GALT and liver**

**Microbiome**

- Increased IL-10 producing T-regs

**Generation of Short Chain Fatty Acids**
Hygiene (now microbial exposure) hypothesis

ALLERGY
Th-2 IL-4↑

INFECTION
Th-1 IGN-γ↑
Early exposure to infections and wheeze phenotype.

Time trends in infectious diseases and immune disorders

Burr ML et al. Thorax 2006;61:296-9
Immunisations and allergic sensitisation in high risk infants with eczema

- Cumulative doses of any vaccines inversely related to eczema severity, p=0.01
- Varicella immunisation inversely related to total IgE and eczema severity.
  OR 0.27 (0.08-0.87)
- BCG (<age 1y) inversely related to sensitisation;
  inhalant OR 0.25 (0.10-0.64)
  food OR 0.25 (0.09-0.67)

Gruber et al Allergy 2008;63:1464-72
Antibiotics in pregnancy and asthma

Number of antibiotic prescriptions in pregnancy

McKeever et al AJRCCM 2002;166:827-832
Caesarean section and asthma.

Roduit et al Thorax 2009;64:107-113

OR
(+/- 95%CI)
for asthma

2 allergic parents
No allergic parent
Farming environments protect against allergy?
The farming effect explained by unpasteurised milk!

Is this effect due to the presence of bacteria and/or prebiotic oligosaccharides in other words a synbiotic, or to other immune modulators which have sequence homology and similar activity to human milk equivalents?
Human milk constituents

- **Lactose**: 53-61 g/l
- **Fat**: 30-50 g/l
- **Oligosaccharides**: 10-12 g/l
- **Protein**: 8-10 g/l
- **Microbiome**: A soup of immune modulators
No doubt “breast is best”

If 37,000 mother infant pairs does not give a significant answer, nothing will!

Problems in gathering evidence

• Unethical to do a controlled trial
• What constitutes breast feeding?
  • Exclusive
  • Predominantly
  • Partial
• What constitutes allergic disease?
  • Positive skin tests
  • Eczema
  • Wheezing
• Confounding factors
• Reverse causation

Matheson, Allen and Tang
Clin Exp. Allergy
2012;42:827-51
The only controlled trial

• Promotion of breast feeding intervention trial (PROBIT) in Belarus
• Over 17,000 mother infant pairs
• Follow-up at 6.5 years
• Intervention group breast feeding at 3 mths 44.3%
• Control group breast feeding at 3 mths 6.4%
PROBIT ISAAC questionnaire results

- Asthma
- Hay fever
- Eczema

Percentage distribution for Intervention and Control groups.
PROBIT skin prick tests results

% of positive reactions for different allergens:
- House mite
- Cat
- Grass pollen
- 1 or more +ve

Comparison between Intervention and Control groups.
Human Milk and Allergic Diseases: An Unsolved Puzzle

Breast feeding = less infection induced wheeze in infancy but more later allergy and asthma

AND

Levels of oligosaccharides and other immune modulators vary in human milk and are affected by many gene/environment interactions

Future studies should identify strategies to improve the allergy protective effects of human milk

Munblit et al. Nutrients 2017, 9, 894; doi:10.3390/nu9080894
Intestinal microbiota - Metagenomics

- Germ free mice have failed tolerance induction in neonatal period with persistent allergic responses. This can be corrected by feeding *a probiotic*
- The microbiota differ in allergically sensitised compared with non-sensitised mice *and humans*
- Transfer of microbiota from an allergic to germ free non-allergic mouse transmits susceptibility to allergic sensitisation
- Caesarean section and pregnancy antibiotics increase the risk of allergy and asthma and farming environment is protective.

*Novas Rivas et al. JACI 2013;131:201-12*
*Tang M. CACI 2017;30:70-74*
Probiotics and eczema prevention.

Kalliomaki et al Lancet 2001:357;1076-9

Bars are 95% CI

Proportion of 2yr olds with eczema (%)

Placebo Lactobacillus GG

Kalliomaki et al Lancet 2001:357;1076-9

No effect on allergy and other studies negative. We need to understand more about gut micro-biotica.

See – Kopp et al Pediatrics 2008;121:850-6
Probiotics for eczema prevention

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Risk Ratio</th>
<th>Risk Ratio</th>
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<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Events</td>
<td>Total</td>
</tr>
<tr>
<td>Abrahamsson 2007</td>
<td>34</td>
<td>95</td>
<td>32</td>
<td>93</td>
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<tr>
<td>Huurre 2008</td>
<td>7</td>
<td>72</td>
<td>12</td>
<td>56</td>
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<td>Kalliomaki 2001</td>
<td>15</td>
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<td>Kopp 2008</td>
<td>19</td>
<td>50</td>
<td>14</td>
<td>44</td>
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<tr>
<td>Kukkonen 2006</td>
<td>120</td>
<td>461</td>
<td>150</td>
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<td>Rautava 2006</td>
<td>4</td>
<td>32</td>
<td>8</td>
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<td>Taylor 2006</td>
<td>38</td>
<td>88</td>
<td>34</td>
<td>87</td>
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<tr>
<td>Wickens 2008a</td>
<td>21</td>
<td>144</td>
<td>20</td>
<td>75</td>
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<tr>
<td>Wickens 2008b</td>
<td>37</td>
<td>152</td>
<td>20</td>
<td>75</td>
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<tr>
<td><strong>Total (95% CI)</strong></td>
<td>1158</td>
<td>1002</td>
<td><strong>100.0%</strong></td>
<td><strong>0.82 [0.67, 1.00]</strong></td>
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<tr>
<td>Total events</td>
<td>295</td>
<td>321</td>
<td></td>
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<tr>
<td>Heterogeneity: Tau² = 0.03; Chi² = 13.44, df = 8 (P = 0.10); I² = 40%</td>
<td></td>
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<tr>
<td>Test for overall effect: Z = 1.94 (P = 0.05)</td>
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</table>
Microbial diversity and eczema

de Vos et al 2013
Pre-biotic oligosaccharides and eczema prevention.
(Moro et al Arch Dis. Child. 2006;91:814-9)

Cumulative incidence of eczema at 6 months (%)

Placebo = Hydrolysed whey formula with maltodextrin.
GOS/FOS = Hydrolysed whey formula with galacto- and fructo-oligosaccharides.

p = 0.014
Prebiotic oligosaccharides for eczema prevention


Cumulative incidence of atopic dermatitis

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence (%)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Placebo (n=24/104)</td>
<td>23.1%</td>
<td>p&lt;0.03 Fisher’s exact</td>
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<tr>
<td>GOS/IcFOS (n=10/102)</td>
<td>9.8%</td>
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Bifidobacteria count (log cfu/g stool)

<table>
<thead>
<tr>
<th>Group</th>
<th>Count (log cfu/g stool)</th>
<th>p-value</th>
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<tr>
<td>Placebo (n=44)</td>
<td>8.7</td>
<td>P&lt;0.001 Mann Witney U-test</td>
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<tr>
<td>GOS/IcFOS (n=50)</td>
<td>10.3</td>
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</table>
Oligosaccharides and atopy by 2 years

Arslanoglu et al., 2008, J. of Nutr. 138:1091-1095

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[Graph showing cumulative incidence (%) for atopic dermatitis, recurrent wheezing, and allergic urticaria with comparison between Placebo and GOS/FOS treatments. Significant differences marked with asterisks.]
Pre-biotic oligosaccharides and eczema free survival

Gruber et al JACI 2010;126:791-7
The PATCH trial

A double-blind, randomized, controlled, parallel-group, intervention trial evaluating the effects of a partial whey hydrolysate with added galacto- and fructo-oligosaccharides (pHF-OS) on the cumulative incidence of allergic manifestations in infants at high risk of developing allergic disease in early life.


Wopereis et al Intestinal Microbiota in Infants at High-risk for Allergy: Effects of Prebiotics and Role in Eczema Development. J. Allergy Clin Immunol. 2017 DOI: http://dx.doi.org/10.1016/j.jaci.2017.05.054

This is consistent with the outcomes from the LEAP and EAT studies that early life ingestion of food allergens induces tolerance while skin and airway exposures sensitise.

Perkin M et al. NEJM 2016 DOI 10.1056/NEJMoa1514210
Du Toit et al NEJM 2015 DOI 10.1056/NEJMoa1414850
PATCH trial T-reg cells at 6 mths

CD25 high and FoxP3 high

% Treg

<table>
<thead>
<tr>
<th>Medium</th>
<th>Control</th>
<th>p = 0.0256</th>
<th>Active</th>
<th>p = 0.0251</th>
</tr>
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</table>

p = 0.0256
PATCH trial plasmacytoid dendritic cells at 6 mths

CD 11c low and CD 123w high

Control
Active

p = 0.056
p = 0.0176
Effect of infant milk formula on cow milk IgG1 at 6 mths and 3 yrs.
Association between cow milk IgG1 at 6 mths and cow milk IgE at 3 yrs

CM-IgE negative
CM-IgE >0.35u/ml

p=0.003
Association between cow’s milk (CM)- IgG1 at 6 mths and hen’s egg (HE)- IgE at 3 yrs

CM-IgG1 in PATCH by HE-IgE in PATCH FU

p=0.035

HE – IgE negative

HE – IgE >0.35u/ml
Effect of intervention on cat dander IgE at 3 yrs

cat dander-IgE at 3 years

- CM formula
- pHF-OS

p=0.0461
Relative abundance of major bacterial taxa from PATCH trial at 4 and 26 weeks

- N= 57 controls
- 51 pHF+OS
- 30 breast fed
Temporal patterns of faecal enterobacter in those with and without eczema by 18 months

No Eczema

Eczema
Associations with eczema at 18 months

- Reduced abundance of Parabacteroides and Enterobacter at 4 weeks
- Reduced abundance of lactate utilising Eubacterium and Anaerostipes at 26 weeks
- Increased lactate and reduced butyrate levels
Maternal environment and human milk composition: Influences on offspring atopy outcomes.
Munblit et al. Nutrients 2017, 9(8), 894; doi:10.3390/nu9080894
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