Microbiota, metabolism and immunity: the potential for early-life intervention.

Mick Bailey
Professor of Comparative Immunology,
Bristol University
Bacterial diversity increases with age in human populations.
Succession of microbial communities: OTU-based community structure and composition in the human gut microbiota changes with age, rather than just becomes more complex.

Koenig J E et al. PNAS 2011;108:4578-4585
The ‘hygiene hypothesis’

• Species co-evolve with their microbiota.

• The immune system *requires* interactions with commensal microbiota and with pathogens for it to express appropriate function (‘enteric health’).

• The absence of appropriate interactions predisposes to diseases of immune dysfunction (allergies, autoimmunity, unusual susceptibility to infectious diseases).

• Similarly, appropriate interactions with gut microbiota are required for development of normal metabolic systems.

• The ‘wrong’ interactions can predispose to metabolic disease in later life.
The ‘hygiene hypothesis’

- Early-life colonisation by microbiota can have long-lasting effects on immunity and metabolism, which may be categorised as ‘beneficial’ or ‘detrimental’ under particular circumstances.
- This is as relevant to our domesticated species as to humans.
CD4+ helper T cells require MHCII-restricted presentation of peptides

Antigen-specific IgM, IgG, IgE, IgA

Antigen-non-specific cytokines
Differentiation of CD4+ helper T-cells

CD4+

Th0

IL-4

IL-12

TGFβ

Th2

GATA-3

IL-4

IL-5

IL-13

Parasites

Th1

Tbet

IFN-γ

Intracellular pathogens

Th17

RORγt

IL-17A/F

Bacteria (PMN)

Treg

FoxP3

IL-10

TGFβ

Damping immunity

CD4+CD8+

Transcription factors

GATA-3

Tbet

RORγt

FoxP3

Signature cytokines secreted

IL-4

IFN-γ

IL-17A/F

IL-10

TGFβ

Target

Parasites

Intracellular pathogens

Bacteria (PMN)

Damping immunity
Novel food proteins trigger inappropriate immune responses in early-weaned piglets
The ability to mount appropriate responses is critical for ‘enteric health’ – IgG, IgE or IgA; Th\(_1\), Th\(_2\), Th\(_{17}\) or T\(_\text{reg}\).

The structure of the mucosal immune system determines the efficiency of responses to food, pathogen and commensal microbiota.
Stages in the development of the mucosal immune system of the pig

1. Rudimentary Peyers patches, essentially no mucosal T cells. Limited B-cell repertoire. Few dendritic cells but MHCII on endothelial cells. The newborn pig.

2. Non-specific expansion of B-cells and Peyers patches. Appearance of early, activated T-cells, influx of MHCII+ cells. 1 days to 2 weeks.

3. Appearance of CD4+ T cells. 2 weeks to 4 weeks.

4. Antigen-specific B-cell responses. Appearance of CD8+ T cells. 4 weeks to 6 weeks.
Most of this expansion of the adaptive immune system is driven by microbiota.

Colonisation of newborn, gnotobiotic piglets with defined microbiota results in expansion of the mucosal immune system which replicates, approximately, that in conventional pigs.

Defined colonisation of gnotobiotic piglets expands mucosal SIRPα⁺ DC first, then CD4⁺ T-cells (Inman, 2012, PLoS One)

- Colonised
- Germ-free
T-cell receptor repertoire is not skewed in fully MHC-inbred, colonised, gnotobiotic piglets

Are studies in gnotobiotic mice and piglets relevant to anything that looks vaguely like the real thing?
Is it important? Genetically identical mice from different suppliers have different immune systems dependant on their microbiota

Ivanov et al, 2008 Cell Host and Microbe 4:337
Is it important? Microbiota is ‘imprinted’ before weaning in neonatal piglets

Non-metric, multidimensional scaling analysis

Tighter clustering for siblings than pen mates

4 different litters (weaning + 21 days)
Is it important? Manipulation of the microbiota and the mucosal immune system by rearing macro-environment (GUTWEAN)


Is it important? Variation between commercial farms in development of the immune system may also be attributable to farm-specific microbiota.

Appearance of memory/effector T-cells (CD4+CD8+) with age (Grierson, Banks, Haverson, Bailey)

<table>
<thead>
<tr>
<th>Tests of Between-Subjects Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Variable: CD4+CD8+ / All CD4+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>Type IV Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>145383.169^a</td>
<td>38</td>
<td>3825.873</td>
<td>26.416</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>1872181.253</td>
<td>1</td>
<td>1872181.253</td>
<td>12926.805</td>
<td>.000</td>
</tr>
<tr>
<td>WEEK</td>
<td>121856.891^b</td>
<td>7</td>
<td>17408.127</td>
<td>120.197</td>
<td>.000</td>
</tr>
<tr>
<td>FARM</td>
<td>462.841^b</td>
<td>4</td>
<td>115.710</td>
<td>.799</td>
<td>.526</td>
</tr>
<tr>
<td><strong>WEEK * FARM</strong></td>
<td><strong>14587.177</strong></td>
<td><strong>27</strong></td>
<td><strong>540.266</strong></td>
<td><strong>3.730</strong></td>
<td><strong>.000</strong></td>
</tr>
<tr>
<td>Error</td>
<td>68504.300</td>
<td>473</td>
<td>144.829</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2297958.454</td>
<td>512</td>
<td>444.829</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>213887.469</td>
<td>511</td>
<td>444.829</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .680 (Adjusted R Squared = .654)
b. The Type IV testable hypothesis is not unique.

Estimated Marginal Means of CD4+CD8+ / All CD4+

Non-estimable means are not plotted.
Can we manipulate this directly in conventional animals with complex, highly variable microbiota?
Intestinal Barrier Function

ZO-1

CD45

E-cadherin

MHCIId
Increased epithelial barrier function after ‘probiotic’ administration
Decreased local IgA and IgM synthesis after ‘probiotic’ administration

MLN – mesenteric lymph node

JPP – jejunal Peyers patch

PSI – upper jejunum

DSI – lower jejunum

Cae – caecum

Col – colon

(Lewis et al, in press, BJN)
Probiotic supplementation changes the structure of immuno-metabolic correlations.

Control B. lactis
Mucosal synthesis of immunoglobulins is linked both to probiotic and to weaning diet. The effect of probiotic is different between diets. Merrifield et al, Gut, in press.

Mucosal synthesis of immunoglobulins is linked both to probiotic and to weaning diet. The effect of probiotic is different between diets. Merrifield et al, Gut, in press.
**B. lactis**, diet and Ig synthesis

Merrifield et al, Gut, in press

![Graph showing the effect of B. lactis on pc scores](image-url)
The effect of probiotic on urinary metabolites also differs between diets.

Merrifield et al, Gut, in press.

**Soya** + *B. lactis*

**Egg** - *B. lactis*
The effects of this probiotic on metabolic and immune function differ between weaning diets:

- The effect is far more obvious on piglets weaned onto egg than those weaned onto soya.
- This is despite the fact that egg and soya were fed for only 4 weeks after weaning.
- At time of sampling (12 weeks old), all piglets had been on the same diet, based on fishmeal, for 4 weeks.
1. Microbiota drives establishment of the architecture of the mucosal immune system.

2. (Different patterns of colonisation, as a consequence of different rearing environments, drive development of different mucosal immune systems.)

3. This can be manipulated by diet and/or probiotic (and/or prebiotic) in early life.

4. Birth and weaning seem to be times of particular susceptibility to changes.

5. However, the patterns of change are complex, interact, and are affected by multiple factors.

6. At present, the effects of any specific pre- or probiotic on specific farms are hard to predict.
**Bristol University**  
Mick Bailey  
Paul Bland  
Tom Humphrey  
Steve Cose  
Ross Harley  
Charlotte Inman  
Paniotis Tourlomousis  
Jenny Bailey  
Phil Jones  
Christine Whiting  
Philippa Lait  
Martin Kenny  
Sakon Singha  
Anusha Edwards  
Louisa Rees  
John Tarleton  
Chris Stokes  
Karin Haverson  
Frieda Jurgenson  
Bevis Miller  
Zoe Christoforidou  
Marie Lewis  
Chelsea Hicks  
Cecilia Harris  
Steve Wilson  
Georgina Laycock  
Ben Bradley  
Andy Weale  
Martin Birchall  
Emma Barker  

**Imperial College, London**  
Jeremy Nicholson  
Elaine Holmes  
Claire Merrifield  
Olivier Cloarec  
Jamie Leigh  
Pam Norton  
Jeremy Morgan  
Adrian Smith  
Mark Stevens  
Pauline Vandiemen  

**Roslin Institute**  
Liz Glass  
Steve Bishop  
Mary Clapperton  
Alan Archibald  

**Aberdeen University**  
Denise Kelly  
Imke Mulder  
Bettina Schmidt  

**Nestec**  
Adrian Zuercher  
Swantje Duncker  
Annick Mercenier  

**J.S.R.**  
Annabelle Hoste  

**Wageningen University**  
Hauke Smidt  
Odette Perez  
Sergei Konstantinov  

**University of Bristol**  

**BPEX**