The ‘Developmental Origins’ of the microbiome

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School of Paediatrics & Child Health, UWA

Overview

• Microbial ecosystems
  - in the evolution and function of human health
• ‘Developmental origins’ of the microbiome
  - Modifying factors
  - Implication for health and disease
• Strategies to promote microbial diversity
  - The role of prebiotics and probiotics
  - Current recommendations
  - Future prospects

Our remarkable and unique world…

…provides a perfect environment for life.

‘Life’ began with simple unicellular organisms…

…similar to primitive life forms today (Archaea).

Disclosures

• President – DOHaD Society (ANZ)
• Chair - WUN-in-FLAME network
• Director - World Allergy Organisation
• Co-Director ORIGINS Project
• Advisory Boards
  - NNI, Danone
  - Speakers fees (ALK, NNI, Danone)
• Author: ‘Allergy Epidemic’, ‘The Calling’ and ‘Origins’

‘Getting the early life origins message out’

All author royalties donated to research

New book
(inflammation is a central theme)

On Amazon, Kindle or direct from publisher

All author royalties donated to research

WEB orders http://uwap.uwa.edu.au
EMAIL to order marketing-uwap@uwa.edu.au

All author royalties donated to research

The ‘Developmental Origins’ of the microbiome

Probiotics and prebiotics in disease prevention?

The International Inflammation Network

Probiotics and prebiotics in disease prevention?

The ‘Developmental Origins’ of the microbiome

Probiotics and prebiotics in disease prevention?
Archea and Bacteria (Prokarya) formed the foundation of all life

...and still contribute at least half Earth's biomass.

More complex life co-evolved with microbes in symbiotic mutualism.

Microbes are essential to the biodiversity that sustains all life

Some evidence that microbes may have driven speciation throughout evolution …

...and continue to drive host physiology, function and behaviour.

Vertebrates evolved complex ‘adaptive’ immune systems: promote symbiosis

- Not just for ‘better defense’
- Selective promotion of beneficial microbes

Bacterial ‘jumping genes’ inserted into host DNA have contributed to evolution of the immune system

Many vast and diverse eco-systems cover all our inner and outer surfaces

“Humans are like a mobile warm blooded coral-reef, home to vast numbers of microbial ecosystems rich in biodiversity”

Highly vulnerable to subtle changes in the environment

Many diverse ecosystems
Variations in microbial diversity with different human habitats

Shannon Diversity Index

Also see variations in diversity of molecular signals

We are a composite of microbes and human cells: ‘We are more microbial than human’

Humans harbour >100 trillion bacteria
- 90% of our cells are microbial
- 99% of our genes are microbial
- 99% of our genetic material subject to ‘rapid’ change in response to the environment (an important therapeutic target)

Critical role in function of virtually all systems

<table>
<thead>
<tr>
<th>Immune development</th>
<th>Brain development</th>
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<tbody>
<tr>
<td>Barrier defence</td>
<td>Neurotransmitters</td>
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<tr>
<td>Metabolism</td>
<td>Mood</td>
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<td>Cardiovascular health</td>
<td>Behaviour</td>
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<td>Anti-inflammatory</td>
<td>Appetite</td>
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<td>Digestion</td>
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Dysbiosis: can adversely affect all of these body functions

Role of declining biodiversity in the NCD pandemic?

Characterised by immune dysregulation and low-grade systemic inflammation

Effect of dysbiosis on the immune system?

Living in a ‘new age’ of chronic inflammation

Impact of modern environmental change on ‘immune health’

Effects apparent in very early childhood

Increase in early-onset NCDs:

Early environmental impact on health and development

Inflammation and immune dysregulation are key factors in all of these conditions
Immune function has a fundamental effect on all systems and functions.

Any factor that influences our immune health can have multisystem effects.

Links to virtually all inflammatory diseases.

Reduced measures of diversity linked to poor health.

Immune function has a fundamental effect on all systems and functions. Any factor that influences our immune health can have multisystem effects.

Links to virtually all inflammatory diseases.

Transplanting bacteria: can change disease risk.

Differences in gut bacteria of obese and lean people.

In animals: changing the gut bacteria changes weight gain, brain and immune development, heart disease and diabetes risk and longevity.

Allergy: reduced diversity predates the disease.

In the first month reduced diversity of life associated with:

- Increased risk of eczema
- Allergic sensitization
- Blood eosinophils
- Allergic rhinitis
- Increased asthma

Strongly suggests early effects on the immune system.

Development is complex, beautiful, and resilient.

...with an inbuilt capacity to adapt to different conditions we might encounter.

The critical importance of a ‘developmental’ perspective.
A long road to maturity and a lot can happen along the way. Early challenges can steer us from our predestined course. Subtle shifts in structure, physiology and behaviour can risk of later disease (NCDs).

Examples of diseases that are developmentally programmed:

- Food allergy, eczema
- Asthma, allergic rhinitis
- Obesity and metabolic disease (diabetes)
- Autism, learning and behavioural disorders
- Depression and anxiety
- Prostate, lung cancer
- Alzheimer’s, Parkinson’s

The effects of the early exposures can last a lifetime. Influence development and function of all organ systems and risk of both early and late onset NCDs. Early events also shape microbiome patterns. Variations associated with exposures and disease patterns.

Changes in our developmental trajectory. Early events can have lasting effects on patterns of gene expression. Through epigenetic changes in gene expression (adaptive vs maladaptive). Short-term adaptations may have long term consequences: disease predisposition.

Early events also shape microbiome patterns. Variations associated with exposures and disease patterns.

Unique microbial ‘finger print’ by adulthood

- Significant individual variability
- Modifiable by external factors (diet, infection etc.)
- Once established: Relatively stable across time

Importance of early influences in establishment of these patterns

Establishing our microbiome
---
Early influences and Developmental patterns

This relationship begins before birth

“The womb is not sterile after all”

- Microbes detected in placenta and fetal tissues in normal pregnancies
- Antenatal source of immunostimulation
- Patterns reflect maternal health, microbiome, diet and environment

Significant capacity to shape many aspects of development

How and where we arrive has a major impact on our microbiota

Delivery mode is a key driver of gut microbiota assembly

Vaginal delivery: Early infant gut colonizers normally resemble mother’s vaginal flora

C-section: altered composition
- less Bacteroides and Bifidobacterium
- Increased skin/mouth bacteria

Breastfeeding also has a major effect on developing microbiota

Breast feeding: major determinant of microbial communities at 12 months (Bifidobacterium and Lactobacillus) vs non-breast fed (more Clostridial);

Life-long legacy effect: persistent differences in adult community types2 and persistent immune effects3.

Starting solid foods: effects not major until breast-feeding ceased

Breastfeeding rather than introduction of solid foods is the dominant factor.
Instability of the early microbiome

Microbiome stability improves with age

- Early Gut flora unstable <3yrs,
- intra-individual stability increases with age despite inter-individual variability

Role for early intervention

Overuse of antibiotics: permanent changes

Evidence that beneficial commensals do not recover completely from antibiotics and replaced by resistant organisms

Concerns focus on 'bacterial resistance'  
— but permanent changes to our protective flora could be more serious

Impact greater in early life before microbiota become established

Overuse of antibiotics: far greater impact than we know

Blaser et al. Nature 2011; 476:393-394

Bacteria have lived with multi-cellular organisms for over 1 billion years

Each 'cleaner' generation may be starting life with a smaller endowment of ancient microbes and than the last

We don’t really even know what we have lost

Effects of eating foods contaminated with low levels of antibiotics?

Murine models:

Low doses of antibiotics in early life (akin to contaminating levels)

- Altered gut flora
- Metabolic effects
- 10-15% increase in weight gain

Implicated in the rising risk of obesity, diabetes, heart disease, allergy and other immune diseases, neurocognitive disorders: multisystem effects

Chu Nature 2012 488: 622-626

Many factors adversely affecting establishment and maintenance of the human microbiome

Diet
- Stress
- Vitamin D
Nature

C-section
Hospital birth
Breastfeeding
Antibiotics
NSAIDS

What can we do to restore the balance?
Role of probiotics and prebiotics -- what do we recommend?

Systematic review of studies: Probiotics for preventing allergy

- Over 15 studies: different protocols, different strains and different outcomes
- Collectively (meta-analyses): Reduction in eczema by 21% (RR: 0.79; 95% CI: 0.71–0.88)
- Less consistent effects on other allergic outcomes, ongoing follow-up.

“Probiotics may be effective in the primary prevention of atopy (mainly eczema).”

Evidence of eczema reduction


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<thead>
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Greatest effects if mixed strains were used: 0.58 (95% CI: 0.44, 0.76).

Meta-analysis: allergic sensitisation

Elazab et al. Pediatrics 2013;132:e666–e676

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RRR: 0.69 (95% CI: 0.62, 0.78).

Effects when prenatally and postnatally
RR: 0.88 (95% CI: 0.78 to 0.99; P = .035)
but not when given only postnatally
AND no effect on asthma or allergic rhinitis

Meta-analysis: asthma and wheeze

Elazab et al. Pediatrics 2013;132:e666–e676

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No effects on asthma/wheeze
RR: 0.96 (95% CI: 0.85, 1.07).

Progress in developing recommendations?
Until 2015: NO specific recommendations from key global organisations1-4

- Pregnancy: No specific recommendations on the use of probiotics.
- Lactation: No recommendations.
- Infants: No recommendations.

But comment (by WGO) “the strongest evidence is for the prevention of atopic dermatitis when certain probiotics are administered to pregnant mothers and newborns up to 6 months of age”15

Taking the GRADE approach

Clinician and Patient focused recommendations
- Recommendation and strength
- Benefits and harms
  Balancing desirable and undesirable consequences
- Confidence of the estimate
  Quality of evidence
- Preferences and values
- Resources
- Rationale

Establised in 2000 and widely adopted (McMaster University)

Desirable consequences

Modest reduction in risk of eczema in children with probiotic supplementation during pregnancy only and/or during breastfeeding and/or to the infant.

Reduction in eczema:

<table>
<thead>
<tr>
<th>PROBIOTICS</th>
<th>Number</th>
<th>Difference (per 100) (95%CI)</th>
<th>Relative effect (RR) (95%CI)</th>
<th>Certainty of the evidence (GRADE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>n=1520</td>
<td>9 fewer per 100</td>
<td>RR 0.72 (0.62 to 0.86)</td>
<td>VERY LOW</td>
</tr>
<tr>
<td>Lactation</td>
<td>N=573</td>
<td>16 fewer per 100</td>
<td>RR 0.58 (0.47 to 0.72)</td>
<td>VERY LOW</td>
</tr>
<tr>
<td>Infants</td>
<td>N=1614</td>
<td>5 fewer per 100</td>
<td>RR 0.82 (0.7 to 0.96)</td>
<td>MODERATE</td>
</tr>
</tbody>
</table>

For all outcomes

Probiotics in pregnant women: infant outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>With</th>
<th>Without</th>
<th>Difference (per 100) (95%CI)</th>
<th>Relative effect (RR)</th>
<th>Certainty of the evidence (GRADE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema (follow-up 1 to 5 years)</td>
<td>365/1520</td>
<td>484/1515</td>
<td>9 fewer per 100</td>
<td>RR 0.71 (0.64 to 0.78)</td>
<td>VERY LOW</td>
</tr>
<tr>
<td>Asthma/urticaria (follow-up 2 to 7 years)</td>
<td>143/982</td>
<td>139/982</td>
<td>0 fewer per 100</td>
<td>RR 0.97 (0.93 to 1.02)</td>
<td>VERY LOW</td>
</tr>
<tr>
<td>Food allergy (follow-up 1 to 5 years)</td>
<td>36/279</td>
<td>41/284</td>
<td>9 fewer per 100</td>
<td>RR 1.08 (0.97 to 1.20)</td>
<td>VERY LOW</td>
</tr>
<tr>
<td>Adverse effects</td>
<td>103/294</td>
<td>88/397</td>
<td>9 fewer per 100</td>
<td>RR 1.03 (0.88 to 1.19)</td>
<td>VERY LOW</td>
</tr>
</tbody>
</table>

For all outcomes

Probiotics in lactating women: infant outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>With</th>
<th>Without</th>
<th>Difference (per 100) (95%CI)</th>
<th>Relative effect (RR)</th>
<th>Certainty of the evidence (GRADE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema (follow-up 6 months)</td>
<td>129/707</td>
<td>213/746</td>
<td>16 fewer per 100</td>
<td>RR 0.58 (0.51 to 0.68)</td>
<td>VERY LOW</td>
</tr>
<tr>
<td>Asthma/urticaria (follow-up 1 to 4 years)</td>
<td>33/256</td>
<td>26/286</td>
<td>5 fewer per 100</td>
<td>RR 1.05 (0.91 to 1.21)</td>
<td>MODERATE</td>
</tr>
<tr>
<td>Food allergy (follow-up 1 to 4 years)</td>
<td>7/82</td>
<td>5/85</td>
<td>5 fewer per 100</td>
<td>RR 1.7 (0.98 to 2.54)</td>
<td>VERY LOW</td>
</tr>
<tr>
<td>Adverse effects</td>
<td>3/79</td>
<td>4/79</td>
<td>5 fewer per 100</td>
<td>RR 1.17 (0.88 to 1.55)</td>
<td>VERY LOW</td>
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</table>
Probiotics in infants: infant outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>With</th>
<th>Without</th>
<th>Difference (per 100) (95%CI)</th>
<th>Relative effect (95%CI)</th>
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<tbody>
<tr>
<td>Eczema (follow-up 0.5 to 6 years)</td>
<td>373/1607 (23.2%)</td>
<td>457/1614 (28.3%)</td>
<td>5 fewer per 100 (RR 0.80 (0.7 to 0.90))</td>
<td>MODERATE</td>
<td></td>
</tr>
<tr>
<td>Asthma/wheezing (follow-up 0 to 6 months)</td>
<td>153/1117 (13.7%)</td>
<td>171/1136 (15.1%)</td>
<td>15 fewer per 1000 (RR 0.9 (0.68 to 1.2))</td>
<td>LOW</td>
<td></td>
</tr>
<tr>
<td>Food allergy (follow-up 1 to 6 years)</td>
<td>29/323 (9%)</td>
<td>33/324 (10.3%)</td>
<td>1 fewer per 100 (RR 0.9 (0.57 to 1.2))</td>
<td>VERY LOW</td>
<td></td>
</tr>
<tr>
<td>Adverse effects (follow-up 4 to 24 months)</td>
<td>116/688 (28.9%)</td>
<td>114/684 (27.1%)</td>
<td>2 more per 100 (RR 1.07 (0.71 to 1.53))</td>
<td>VERY LOW</td>
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</tbody>
</table>

The WAO guideline panel suggests using probiotics in pregnant and lactating women, and in infants when there is high risk* of allergy in the children.

* High risk for allergy in a child = a biological parent or sibling with existing or history allergic disease.

The WAO guideline panel considered the risk of adverse effects low.

- The burden of taking daily probiotic supplement is low.
- Any estimate of potential adverse effects was of low certainty
- Certain preparations of probiotics might not be acceptable to some children because of unpleasant taste.

The Recommendation

Considering all critical outcomes: there is a net benefit resulting primarily from prevention of eczema

This recommendation places a relatively high value on prevention of eczema in children, and a relatively lower value on avoiding possible adverse effects.

The WAO guideline panel suggests using probiotics in pregnant and lactating women, and in infants when there is high risk* of allergy in the children.

In Practice?

- The timing, duration, and choice of probiotic (strain and dose) are not specified in the WAO guidelines.
- Probiotics were given in the last four to six weeks of pregnancy in most of the studies reviewed.
- But there was much greater variability in timing and duration of postnatal therapy in the infant and/or breastfeeding mother.
- In addition, the only probiotic strain with reproducible data is Lactobacillus rhamnosus GG (LGG).

More research needed: to define optimal strains, timing, and other interactions

What? When? How?

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- Probiotics were given in the last four to six weeks of pregnancy in most of the studies reviewed.
- But there was much greater variability in timing and duration of postnatal therapy in the infant and/or breastfeeding mother.
- In addition, the only probiotic strain with reproducible data is Lactobacillus rhamnosus GG (LGG).
In the end:

Our approach — “The data suggest that there is a modest preventive effect of probiotics on the development of eczema, but not other atopic diseases, in at-risk infants (defined as presence of a biologic parent or sibling with asthma, allergic rhinitis, eczema, or food allergy. However, the great heterogeneity of the studies makes it difficult to advise on specifics regarding therapy (eg, strains, dose, timing, and duration).

Thus, we do not suggest giving probiotics during pregnancy, lactation, and infancy for the prevention of eczema (Grade 2C).

“We do not discourage this approach, however, if parents express interest, given the low risk of adverse events. Parents who chose this approach should first discuss it with the appropriate clinician”.

The panel met in Jan 2015

World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Prebiotics – coming

Position Article and Guidelines  Open Access

World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Prebiotics – coming

Antropos Focm3, Background, Carls Curdo-Garcia4, Vagno s, Zabihian Atkinson5, Amar Algarni6, Kirsten Bay7, Wendy Buhb8, Giorgio Di Camerino9, Motoko Tobin10, Shinya Cachi11, Ross Katermai12, Ben Wu Lee13, Hugh W. Lean14, Susan Preziosi15, Mike J Mau12, Larry Rosen12, Hugh Sampson15, Michael Spiegel16, Luigi Spinnato17, Andrea Veenstra18, Susan Wolfram15, Juan Jose Vesper Huerta19, Jan L. Broek20, and I. Kodak 12 Schuurna21

**Finding are pending - No other organisations have recommendations at present**

Results of new studies expected this year

Anticipate WAO recommendations soon (late 2015)

Roles prebiotics?

Children: prebiotics show early promise in allergy prevention

(Still limited studies)

RCT in high risk infants (n=259)1,2

- GOS/FOS mix (FF)
- Effects on colonisation (increased bif)
- Reduced eczema (6 mo and 2 yrs)
- Reduced recurrent wheeze / urticaria

RCT in low risk infants (n=830)3

- stool consistency and colonisation
- also showed reduced eczema

1 Moro et al. ASCI 2006
3 Gruber et al. JACI 2010

Currently no recommendations

More of this

UNHEALTHY

Less of this

HEALTHY

“Currently the best way to improve our gut health is a healthy balanced diet – rich in fibre, fresh fruit and vegetables – and this is likely to have many other benefits for mother and child”

We cant recommend specific products and a whole food approach is likely to have more general health benefits.
Food for thought: changing our bacteria by changing what we eat

Who is there is important, but what they are doing may matter more!

Diet contributes to both ‘who’ and ‘what’

Gut microbiome responds rapidly to dietary change

Metabolites cross the placenta and into breast milk, to influence gene expression in offspring

SCFA metabolites

Dietary change driving altered microbiota biodiversity

Effects on immune and metabolic regulation

Immune and metabolic effects of the microbiome through metabolic sensing pathways begin in utero
Fibre in pregnant mice: prevents allergy in offspring

Prebiotic effects – modulation of maternal microflora

Thorburn and Mackay (et al.) Nature Comm 2015

High fibre chow or SCFA

Offspring of Pregnant Mice fed high fibre diets or SCFA (acetate) have reduced allergic responses

Human trials now underway

The microbiome: a new frontier for small molecule discovery

Enormous potential for new therapeutic targets and ‘drugs’

Garber Nature Technology 2015:33:228-231

Microbiome has evolved to interact with humans:

- Produce 1000’s ‘drug-like’ molecules.
- Many receptors and effects still unknown.

Potential for discovery much greater than searching the ‘external’ environment.

Search for new metabolites and gene products for millions of microbial targets

Small molecules:
- Allow non-lethal modulation of the microbiome (an ‘alternative’ antibiotic).
- Capacity to influence many aspects of human biology without ‘systemic’ delivery.

Human genome only provides 20,000 targets: microbiome provides vast new possibilities

‘Targeted approach’ more predictable (and profitable) than pre/probiotic ‘black-box’

Pharma perspective:
- Food ingredients, fibre and SCFA are not a ‘viable business plan’ for patent claims unless part of small molecules cocktails.

Recent surge in investment, trials, preclinical and discovery research in this field
Tension between holistic vs reductionist approaches:

Natural-holistic
Lifestyle and Diet
Balance in complexity (prevention)

Technology-targeted
Driven by patents, profits and Pharma (treatment)

In summary...

Impact of human activity on ‘health’ of the environment

Adverse effects on our own health

"Disrupting the balance"
Biodiversity
Microbial balance
Pollution
Food, air & water quality
Global warming

An unhealthy relationship

Early effects on the human microbiome: long term consequences

Complex large-scale environmental change linked to reduced biodiversity:

Agricultural practices
Livestock and bacteria
Air Pollutants
Water contaminants
Processed /sterile foods

Contribute to ‘dysbiotic drift’ in environment and humans

We have forgotten that we need nature more than nature needs us

‘Humans vs Nature’

We must find ways of restoring ‘balance’: for our own sake

Our health depends on the health of the environment

…and the biodiversity that sustains all life
Early interventions can reduce the risk of both early and later NCDs.

The impact of the early microbiome and immune health underestimated.

Effect of the early environment lasts a lifetime.

We need to start by changing the menu.

Food is central in both the origins and prevention of disease.

By changing our food we can change our gut bacteria, our genes and our metabolism.

Replace processed foods in your diet.

Fat fresh. Eat local. Start today.

More fresh vegetables, whole grains, nuts, fruit, legumes.

Slow Food

“...to change the world, you have to change the menu first”

Solutions with personal, social, economic and community benefits - are possible.

You can make a difference.

Change starts with us: and the choices we make.

Every choice sends a ‘signal’

- To your body
- To your family
- To your community
- To businesses and industry
- To politicians and policy

Our food choices impact the health of the environment.
Taking responsibility at every level

Many of today's problems are the consequences of yesterday's decisions.

Government
Business
Responsibility is needed at every level
Individuals

Health of tomorrow will depend on what we do today.

Advocates for long-range vision and long term commitments

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