Gene-Environment Interaction in Chronic Inflammatory Disease

Pre- and postnatal environment

opportunity for early immuno-education and -programming
The fetal origins of adult disease: Barker Hypothesis

is strongly associated with

Reduced birthweight and infant weight

Coronary heart disease

Study population:
- 5654 men
- born in East Herfordshire
- between 1911 and 1930

Dr. David Barker

D.J.P. Barker; British Medical Journal, 1992
D.J.P. Barker; The Lancet 1989
The fetal origins of adult disease: Barker Hypothesis

Reduced birthweight and infant weight is strongly associated with Coronary heart disease

Study population:
- 5654 men
- born in East Herfordshire
- between 1911 and 1930

- Hypertension
- Stroke
- T2D
- Osteoporosis
- Aging
- Breast and ovarian cancer

Dr. David Barker

D.J.P. Barker; British Medical Journal, 1992
D.J.P. Barker; The Lancet 1989
The Dutch famine 1944/45
Prenatal nutrition and the disease in subsequent generations
The Dutch famine birth cohort

Mother (F0)  
1945/46  
birth F1  
1944/45  
Winter  
1970s  
1990s  
Energy uptake  

500 kcal  
2000 kcal  
Analysis of phenotypes  
~ 1990  
~ 1975
Prenatal nutrition and the disease in subsequent generations
The Dutch famine birth cohort

Mother (F0) → Fetus F1 → Energy uptake → 1945/46 → birth F1 → 1970s → 1990s → analysis of phenotypes ~ 1990

2000 kcal
500 kcal

Winter 1944/45

Early
- Obesity
  (Ravelli et al., 1999)
- Schizophrenia
  (Susser et al., 1992)
- Coronary heart disease
  (Roseboom et al., 2000)
- Breast cancer
  (Painter et al., 2006)

Late
- Impaired glucose tolerance
  (Ravelli et al., 1998)
- Major affective disorders
  (Brown et al., 2000)

Germ cells F2

Obesity
(Painter et al., 2008)
Maternal as well as grandmaternal smoking increases asthma risk in early childhood

*Adjusted for race/ethnicity, gestational age and second hand smoke

Li et al, Chest, 2005
Maternal as well as grandmaternal smoking increases asthma risk in early childhood

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Li et al, Chest, 2005
Maternal as well as grandmaternal smoking increases asthma risk in early childhood.

Adjusted for race/ethnicity, gestational age and second hand smoke

*OR  1.0 *  1.3 *  1.8 *

Li et al, Chest, 2005
Maternal as well as grandmaternal smoking increases asthma risk in early childhood

* Adjusted for race/ethnicity, gestational age and second hand smoke

Li et al, Chest, 2005
Commensal bacteria promote development of respiratory mucosal immune system

Commensal bacteria promote development of respiratory mucosal immune system

Dysbiosis and chronic allergic inflammation of the respiratory mucosa

Loss of clinical and immunological tolerance

- Hygiene hypothesis
  - Lack of infectious microbes in early childhood
  - Altered exposure to environmental microbes
  - Examples:
    - having older siblings
    - frequent viral infections
    - anthroposophical life style
    - early day care
    - traditional farming
Traditional farming and early immuno-education

Courtesy: Erika von Mutius

ALEX-Study (1998 – 2002)
EU-6FWP PASTURE (2002 – 2007)
EU-7FWP PRO-IMMUNE (2008 – 2011)
Cause – effect relationship between exposure-immune response and clinical phenotype

Environmental exposure / Nutrition

- local/mucosal
- systemic

- innate
- adaptive
- effector

responses

clinical phenotype
Inverse relationship between endotoxin-load and atopic phenotypes

Braun-Fahrländer et al.
NEJM, 2002
Farmers express higher levels of CD14 and TLR-2, but not TLR-4

Lauener et al, Lancet 2002
Prenatal farm exposure relates to the expression level of TLRs at school age

- cross-sectional (PARSIFAL)
- subsample of n = 322 children
- adjusted for age, sex, family history of atopy, parental education, environmental tobacco smoking, maternal smoking during pregnancy, number of older siblings, contact with pets ever, child’s current exposure to a farming lifestyle, child’s exposure to farm animals, and predominant farm milk consumption of the child

Ege et al, JACI 2006
Inverse relationship between in-vitro IFN-\(\gamma\) and IL-10 production and in-door endotoxin load.

- stimulation of PBMC with 1 µg/ml LPS
- collection of supernatants after 24 h

Braun-Fahrländer et al. NEJM, 2002
Inverse relationship between in-vitro TNF-α and IL-12 production and in-door endotoxin load

Braun-Fahrländer et al. NEJM, 2002
## Examples of bacterial species inversly related to childhood asthma and atopy

<table>
<thead>
<tr>
<th>Health outcome</th>
<th>Bacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physician’s diagnosis of asthma</td>
<td><em>Corynebacterium sp</em></td>
</tr>
<tr>
<td></td>
<td><em>Jeotgalicoccus sp</em></td>
</tr>
<tr>
<td></td>
<td><em>Gardnerella vaginalis</em></td>
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<tr>
<td></td>
<td><em>Lactobacillus curvatus, saker</em></td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus sciuri</em></td>
</tr>
<tr>
<td>Atopic sensitization</td>
<td><em>Lactobacillus iners</em></td>
</tr>
<tr>
<td></td>
<td><em>Acinetobacter lwoffii</em></td>
</tr>
</tbody>
</table>

### Environmental microbes with asthma protective properties

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter lwoffii</td>
<td>TH1 / IFNγ ↑</td>
<td>1. Conrad et al, JEM 2009</td>
</tr>
<tr>
<td>Staphylococcus sciuri</td>
<td>T-cell activation ↓</td>
<td>2. Hagner-Benes et al, submitted</td>
</tr>
<tr>
<td>Lactococcus lactis</td>
<td>TH2 ↓ (IFNγ) ↑</td>
<td>3. Debarry et al, JACI 2007</td>
</tr>
<tr>
<td>Bacillus licheniformis</td>
<td>TH1 pathology</td>
<td>4. Vogel et al, JACI 2008</td>
</tr>
<tr>
<td>LPS</td>
<td>IL-12 ↑ TH1 ↑</td>
<td></td>
</tr>
</tbody>
</table>

1. Conrad et al, JEM 2009
2. Hagner-Benes et al, submitted
3. Debarry et al, JACI 2007
4. Vogel et al, JACI 2008
Mouse model of prenatal immuno-modulation - proof of principle -

A. lwoffii F78 exposure

Mating  Pregnancy  Birth

Mother

Offspring

days  -10  0  20

OVA sensitization  OVA aerosol challenge

Phenotype

25  39  44  46  47  48
Maternal signalling pathways of asthma protection
Maternal signalling pathways of asthma protection

Intact signalling through pattern recognition receptors (e.g. TLRs)
Toll-like receptors and their ligands
Prenatal *A. lwoffii* F78 exposure prevents the allergic phenotype

**Goblet cells**

<table>
<thead>
<tr>
<th>Maternal exposure</th>
<th>PBS</th>
<th>PBS</th>
<th><em>A. lwoffii</em></th>
<th><em>A. lwoffii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring sensitization</td>
<td>PBS</td>
<td>OVA</td>
<td>PBS</td>
<td>OVA</td>
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</table>

**Eosinophils**

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>PBS</th>
<th><em>A. lwoffii</em></th>
<th><em>A. lwoffii</em></th>
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<td>nd</td>
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**Airway reactivity**

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>PBS</th>
<th><em>A. lwoffii</em></th>
<th><em>A. lwoffii</em></th>
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</table>

Intact maternal TLR-signalling is required for allergy protection

Maternal signalling pathways of asthma protection

Intact signalling through pattern recognition receptors (e.g. TLRs)

Mild systemic pro-inflammatory response
The acinetobacter lwoffii medicated asthma protection depends on IL-6

Kesper et al, 2014
The acinetobacter lwofii medicated asthma protection depends on IL-6

Kesper et al, 2014
Mucosal Exposure to *A. lwoffii* triggers a pro-inflammation response.
Maternal signalling pathways of asthma protection

Intact signalling through pattern recognition receptors (e.g. TLRs)

Mild systemic pro-inflammatory response

Placenta: altered expression of inflammatory genes
Transmaternal asthma protection - importance of microbial exposure -

The early beginnings

Catherine Hawryłowicz & Kimuli Ryanna

RESEARCH HIGHLIGHTS

Prenatal protection through TLRs

Soothing signals: transplacental transmission of resistance to asthma and allergy

Patrick G. Holt and Deborah H. Strickland
Epigenetics: Linking Environment and Genes

DNA

RNA

Protein

Epigenetics: Linking Environment and Genes

DNA

RNA polymerase
messenger RNA

RNA

linear protein

Protein

folded protein

Some genes are open and accessible, others are closed

Epigenetics: Linking Environment and Genes

Some genes are open and accessible, others are closed

Determined by environmental triggers (e.g. food, toxins, microbes, allergens, stress…)

Some genes are open and accessible, others are closed

Determined by environmental triggers (e.g. food, toxins, microbes, allergens, stress…)

Regulated by epigenetic mechanisms

Epigenetics: Linking Environment and Genes
The two main components of the epigenetic code

DNA methylation
~ 30 Mill CpG islands

Histone modification
~ 30 Mill nucleosomes

Gene-specific differences – DNA methylation of TH1 and TH2 cells

IFNγ promoter

CNS-1

IL-4 promoter

IL-5 promoter

murine T cell lines

TH1 (LNC2)

TH2 (L1/1)

Brand S, Kesper D et al.
JACI 2012
Histone Modifications of cytokine genes
- proof of concept for functional relevance -

CD4

IL-4
IL-5
CNS-1
INF-γ

H4 ac

opens

INF-γ↑

Brand, Kesper et al., JACI 2012
Histone Modifications of cytokine genes - proof of concept for functional relevance -

Brand, Kesper et al., JACI 2012

CD4

IL-4
IL-5
CNS-1
INF-γ

H4 ac

opens

INF-γ↑

anti-INF-γ treatment or inhibition of histone Acetyltransferase (Garcinol)

prevents A. Lwoffii effects

Brand, Kesper et al., JACI 2012
Regulation of IFN-γ gene expression by DNA methylation – age dependent differences

• The IFNγ locus is hypermethylated at birth specifically in CD4+/CD45RO-

• Decreased methylation in adults corresponds with increased IFNγ production

White et al, J Immunol 2002
Loss of clinical and immunological tolerance

Microbiota hypothesis

- modern/ industrialized life style
  - altered microbial diversity
  - loss of ancient co-evolved microbes
  - altered immune response
  - disease
- (organized) microbial colonization of skin and mucosal surfaces

Biodiversity Hypothesis
Impact of barrier microbes on organ-based inflammation

Maternal Factors
- allergic phenotype
- (intestinal) colonization
- mode of delivery
- breast feeding

- reduced diversity
- dysbiosis early in life (0 ~ 6 mths)

Sensitization (Th2) (+)
Eczema (+)
Food allergy (+/-)
Allergic rhinitis / asthma (?)

### Altered microbiome precedes development of allergic phenotypes

<table>
<thead>
<tr>
<th>Reduced diversity/ altered composition at</th>
<th>Phenotype</th>
<th>Assessed at</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>Eczema</td>
<td>2 years</td>
</tr>
<tr>
<td>day 7</td>
<td>Eczema</td>
<td>12 months</td>
</tr>
<tr>
<td>1 week</td>
<td>Eczema</td>
<td>18 months</td>
</tr>
<tr>
<td>1 week / 12 months</td>
<td>IgE, eos., rhinitis;</td>
<td>up to 6 years</td>
</tr>
<tr>
<td></td>
<td>not asthma, eczema</td>
<td></td>
</tr>
<tr>
<td></td>
<td>asthma</td>
<td></td>
</tr>
</tbody>
</table>

Abrahamsson et al. JACI 2012;129:434-40.
Ismail et al. PAI 2012; 23:674-81.
Wang et al. JACI 2008; 121:129-34.
Bisgaard et al. JACI 2011; 128:646-52.
Inverse relationship between microbial exposure and probability of asthma

A - Bacteria

B - Fungi

Ege et al., NEJM, 2011
## Caesarean delivery: Allergy and asthma risk

<table>
<thead>
<tr>
<th>Outcome</th>
<th>N*</th>
<th>Fixed effects model OR, 95% CI</th>
<th>Random effects model OR, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food allergy/food atopy</td>
<td>6</td>
<td>1.32 (1.12–1.55)</td>
<td>1.45 (1.12–1.86)</td>
</tr>
<tr>
<td>Inhalant atopy</td>
<td>4</td>
<td>1.06 (0.87–1.28)</td>
<td>1.07 (0.82–1.38)</td>
</tr>
<tr>
<td>Eczema/atopic dermatitis</td>
<td>8</td>
<td>1.03 (0.98–1.09)</td>
<td>1.03 (0.98–1.09)</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>7</td>
<td>1.23 (1.12–1.35)</td>
<td>1.24 (1.08–1.43)</td>
</tr>
<tr>
<td>Asthma</td>
<td>13</td>
<td>1.18 (1.11–1.23)</td>
<td>1.18 (1.05–1.32)</td>
</tr>
<tr>
<td>Hospitalization for asthma</td>
<td>7</td>
<td>1.23 (1.18–1.27)</td>
<td>1.21 (1.12–1.31)</td>
</tr>
</tbody>
</table>

Medline 1966-2007
26 studies

Bager et al, Clin Exp Allergy, 2009
Diversity of mother’s body habitats

Mother’s body habitat or Baby’s delivery mode

Dominguez-Bello et al; PNAS 2010