The Interaction Between Gut Microbiome, Immune System and Allergy Outcomes

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Presentation Outline
- DOHaD concept
- Establishment of the gut microbiota
- Gut microbiota and immune development
- Gut microbiota and allergic disease

Hygiene Hypothesis: Allergic and Autoimmune Diseases
- Increased prevalence of immune mediated disorders correlated with reduced prevalence of infectious diseases
- Strachan first posed the Hygiene hypothesis in 1989
- Importance of overall microbial exposure rather than specific infections
  - Since early/mid 19th century,... Reduced exposure to microorganisms which we have lived with since the paleolithic age → Concept of evolved adaptation

Auto-inflammatory Diseases: Common Pathways?

Environment Risk Factors
- Microbial exposures in early life
  - Hygiene hypothesis
  - Intestinal microbiota
- Infant diet
  - Type of food first year of life
  - Timing of exposure to food allergens
- Vitamin D / UV exposure
- Immunomodulatory dietary factors
  - Omega-3 fatty acids
  - Folate?
- Pollutants

Early Life Programming of Disease Risk: DOHaD

- Timely intervention produces substantial risk reduction
- Impact of adult intervention is small
- 9 mos to 24 mos Life course
- Fixed genetic contribution to risk is small

Hanson and Gluckman AJCN 2011

Strachan DP. BMJ 1989;299:1259–60;
Rook GA. Die Exp Immunol; 160:70–76.
Intestinal Microbiota

The most abundant microbial exposure during life is establishment of the gut microbiota... major impact on immune responses

- 100 trillion bacteria
- *Gut Microbiota*
- 60-70% of immune cells
- Surface of approximately 300m²
- 100 million neurons

Establishment of the Intestinal Microbiota

- Gut microbiota evolves rapidly from birth → Relatively stable by 2 years
  - First bacterial communities (seeding bacteria) are acquired from the mother and the environment
  - Diversity increases over the first year and stabilises thereafter
- Microbiota composition is influenced by early life exposures
  - Mode of delivery (cesarean vs vaginal)
  - Maternal microbiota (stress/diet during pregnancy)
  - Breast milk vs formula feeding
  - Diet
  - Sanitation and level of cleanliness
  - Antibiotic therapy
- Stable microbiota is resilient to acute environmental changes
  - Compositional homeostasis

Transitions in Gut Microbiota Composition

Intestinal Microbiota and Immune Development

- The intestinal microbiota plays a crucial role in development of the mucosal and systemic immune system
- Mice bred in germ free conditions have abnormal lymphoid tissues
  - Small underdeveloped Peyer’s Patches, MLNs and spleen
- Mice raised in a germ free environment fail to develop oral tolerance
  - Have persistent and exaggerated Th2 dependent responses to OVA
- These abnormalities of lymphoid tissue structure and oral tolerance can be corrected by seeding the intestine with Bacteroides fragilis but...
  - ONLY if this occurs in the neonatal period

Intestinal Microbiota and Immune Regulation

- Germ free mice (that lack intestinal microbiota)
  - Reduced numbers of FoxP3+ Treg in MLN +/- PP
  - Treg impaired suppressor function and reduced IL10 and TGFβ production
  - Reduced production of IL12, IFNg, IL10; and increased production of IL4

An altered microbiota is associated with increased risk for development of allergic disease

2. Umesaki Y et al. Microbial Immun 1995;9(9): 555-62
Intestinal Microbiota in Allergic and Non-allergic Children

- Children with eczema have altered microbiota 1-3
  - lower counts of bifidobacteria and lactobacilli
  - higher counts of S aureus, C difficile and E coli

- These differences precede the onset of allergic disease 4-6
  - lower counts of bifidobacteria and enterococci at 1 mos
  - lower counts of bifidobacteria and bacteroides at 12 mos
  - more often colonized with S aureus at 6 mos


Intestinal Microbiota in Allergic and Non-allergic Children

- The composition of bifidobacterium flora is also different in children with allergic disease
  - Specific strain differences vary in different locations

- Scandinavia
  - allergic subjects have predominance of B adolescentis (adult-type)
  - healthy infants have predominance of B bifidum and B catenulatum / pseudocatenulatum
  - reduced adhesion to human intestinal mucus
  - induce less IL10 production in vitro

- Japan and New Zealand
  - Allergic subjects higher levels of B bifidum (Japan) and B catenulatum (Japan, NZ)


PEPS cohort: Reduced Microbial Diversity at Day 7 in Infants with Eczema Ever at 12 months

<table>
<thead>
<tr>
<th>Allu1</th>
<th>Sau961</th>
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<tbody>
<tr>
<td>p=0.029</td>
<td>p=0.003</td>
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</table>

Ismail et al. Pediatr Allergy Immunol 2012;23:674-81

Abrahamsson et al. J Allergy Clin Immunology 2012;129:434

Intestinal microbiota in mice susceptible to food allergy

IL4RaF709 mice
- Carry a gain of function mutation in IL4Ra chain
- Susceptibility to experimentally induced food allergy

<table>
<thead>
<tr>
<th>WT + PBS</th>
<th>WT + OVA/CEB</th>
<th>IL4RaF709 + PBS</th>
<th>IL4RaF709 + OVA</th>
<th>IL4RaF709 + OVA/CEB</th>
<th>IL4RaF709 + OVA - CEB11.10^5</th>
<th>IL4RaF709 - CEB11.10^5</th>
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Noval Rivas M et al. JACI 2013;131:201-12

Sensitisation to OVA in IL4RaF709 mice induces a specific microbial signature

Phylum
- Bacteroidetes
- Actinobacteria
- Firmicutes
- Proteobacteria
- Other

The color saturation indicates the degree of difference from the mean value of the sham-treated samples

Noval Rivas M et al. JACI 2013;131:201-22

Intestinal microbiota from sensitised IL4RaF709 mice transmits susceptibility to experimentally induced food allergy

Germ Free WT mice

<table>
<thead>
<tr>
<th>OVA Challenge: 150 mg</th>
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<tbody>
<tr>
<td>Flora from OVA-WT mice</td>
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<tr>
<td>Week 0</td>
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<tr>
<td>Flora from OVA-IL4RaF709 mice</td>
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<tr>
<td>WT GF + WT Flora</td>
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<td>WT GF + WT-IL4RaF709 Flora</td>
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Noval Rivas M et al. JACI 2013;131:201-12
Transfer of Treg cells can prevent food allergy and induces a tolerogenic microbial signature

Compared with mean value of sham-treated mice

Transfer of Treg cells can prevent food allergy and induces a tolerogenic microbial signature

Infants with eczema have reduced Treg capacity in response to TLR stimulation at birth

Infants with eczema have reduced Treg capacity in response to TLR stimulation at birth

iTreg can Influence Microbiota

- iTreg are important for control of unwanted allergic inflammatory responses at mucosal sites
  - Absence of iTreg in CNS1 mice was associated with intestinal and lung Th2 inflammation
- iTreg help maintain a ‘normal’ microbial community in the gut
  - Absence of iTreg in CNS1 mice resulted in altered gut microbial communities
  - enrichment of phylum TM7, genus Alistipes
  - overall decrease in the ratio of Firmicutes to Bacteroidetes

Percentage of total 16S rRNA gene sequences of the Firmicutes and Bacteroidetes phyla in stool from individually housed CNS1 (n=9) and WT (n=6) littermate mice.

Summary

- The rapid rise in non-communicable diseases has occurred as a result of environmental changes associated with the modern lifestyle; in particular, reduced microbial exposures have led to altered intestinal microbial signatures that are associated with allergic disease risk
- The intestinal microbiota plays a critical role in immune development and early life immune programming
- Alterations in the intestinal microbiota in the first weeks of life are associated with development of allergic diseases in later life
- In mouse models, disease risk can be conferred by transfer of disease associated microbial signatures; and conversely, protection against disease can be conferred by favourable microbial signatures
- Infants who develop eczema by 1yr have reduced Treg response to TLR stimulation from the time of birth
- Modulation of the intestinal microbiota in early life may offer a novel approach to prevention or treatment of allergic disease; and may be most beneficial in infants at increased risk of allergic disease