Effect of early life antibiotics on development and later disease?

Martin J Blaser

Departments of Medicine and Microbiology
New York University School of Medicine
Department of Biology, NYU
New York Harbor VA Medical Center
What happens when our early life microbes are perturbed?

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Obesity trends among U.S. children and adolescents*

*Sex- and age-specific BMI > 95th percentile based on CDC growth charts
ANTIBIOTICS
## Outpatient antibiotic use, by age, 2010

<table>
<thead>
<tr>
<th>Patient age group (years)</th>
<th>Number of prescriptions (millions)</th>
<th>Prescriptions /1000 people</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1</td>
<td>16.6</td>
<td><strong>1365</strong></td>
</tr>
<tr>
<td>2 - 9</td>
<td>29.0</td>
<td>1021</td>
</tr>
<tr>
<td>10 - 19</td>
<td>28.9</td>
<td>677</td>
</tr>
<tr>
<td>20 - 39</td>
<td>55.4</td>
<td>669</td>
</tr>
<tr>
<td>40 - 64</td>
<td>81.6</td>
<td>797</td>
</tr>
<tr>
<td>≥ 65</td>
<td>41.1</td>
<td>1020</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>258.0</strong></td>
<td><strong>833</strong></td>
</tr>
</tbody>
</table>

## Cumulative outpatient antibiotic use, by age

<table>
<thead>
<tr>
<th>Patient age group (years)</th>
<th>Number of prescriptions (millions)</th>
<th>Prescriptions /1000 people</th>
<th>Average number of courses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>During period</td>
</tr>
<tr>
<td>0 - 1</td>
<td>16.6</td>
<td><strong>1365</strong></td>
<td><strong>2.73</strong></td>
</tr>
<tr>
<td>2 - 9</td>
<td>29.0</td>
<td>1021</td>
<td><strong>8.17</strong></td>
</tr>
<tr>
<td>10 - 19</td>
<td>28.9</td>
<td>677</td>
<td><strong>6.78</strong></td>
</tr>
<tr>
<td>20 - 39</td>
<td>55.4</td>
<td>669</td>
<td>13.38</td>
</tr>
<tr>
<td>40 - 64</td>
<td>81.6</td>
<td>797</td>
<td>19.93</td>
</tr>
<tr>
<td>≥ 65</td>
<td>41.1</td>
<td>1020</td>
<td>-</td>
</tr>
</tbody>
</table>

Total 258.0 833

Outpatient antibiotic usage rates by region, 2010

- Northeast: 830
- Midwest: 868
- West: 638
- South: 936

National rate: 833/1000 population (258 million courses)

Comparisons between the geography of obesity and antibiotic use, 2010

Adult obesity and antibiotic prescriptions in children, 2011

Source: CDC and CDC (L Hicks et al. CID 2015)
Evolutionary relationships of wild hominids

H. Ochman et al. *PLoS Biology* 2010
When does the adult gut microbiome become established?

Schematic of interaction between a co-evolved colonizing microbe and host

- Robust
- Resilient

Perturbation?

Mother ➔ Child Transfer of Microbes (Modern)

- Effect of maternal exposures
  - Environment
  - Antisepsis
  - Antibiotics
  - Diet
  - Other hosts
  - Epigenetics

- Oral (pre-mastication of food)
- Mammary, through breastfeeding (selection)
- Cutaneous (contact with skin)
- Vaginal (passage through birth canal)

- Dental amalgam
- Bottle feeding
- Early-life antibiotics
- Early/extensive bathing
- Caesarean section

*Nature Reviews Genetics 2012;13:260-70*
The “Disappearing Microbiota” hypothesis

• Beginning in the late 19\textsuperscript{th} century, changing human ecology has dramatically altered the transmission and maintenance of our indigenous microbiota.

• These changes have affected its composition.

• This altered composition affects human physiology, and thus disease risk.

• Loss of ancestral bacteria, usually acquired \textit{early in life}, is especially important, because it affects a developmentally critical stage.

\textit{Lancet} 1997;349: 1020
\textit{Gut} 1998; 453: 721
\textit{EMBO Reports} 2006; 7:956.
\textit{Nature} 2011; 476:393.
The effect of maternal status on the resident microbiota of the next generation

Disappearance of *Helicobacter pylori* in Japanese families

Approximate year of birth

<table>
<thead>
<tr>
<th>Year</th>
<th>% positive</th>
<th>% decline in prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940</td>
<td>68.7</td>
<td>-</td>
</tr>
<tr>
<td>1970</td>
<td>43.4</td>
<td>37</td>
</tr>
<tr>
<td>2000</td>
<td>12.5</td>
<td>71</td>
</tr>
</tbody>
</table>

Grandmothers (244/355)
Mothers (251/578)
Children (101/808)

Adapted from Y. Urita et al. *J Ped Child Health* 2013; 49:394-8
Fecal diversity in four human groups

J Clemente et al.

Science Advances 2015

Maria Gloria Dominguez Bello

J Clemente et al.

Science Advances 2015
What are the consequences of adding exposures in the context of an impacted microbiota?

- Vertical acquisition
- Potential horizontal acquisition

Representation of a conserved microbiota

Cumulative + Early life exposures?
Sub-therapeutic antibiotic treatment (STAT) used for growth promotion of livestock

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Class</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bambermycin</td>
<td>Glycolipid</td>
<td>Cell wall</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>Streptogrammin</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Avilamycin</td>
<td>Orthosomycin</td>
<td>Protein synthesis</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>Cyclic peptide</td>
<td>Cell wall synthesis</td>
</tr>
<tr>
<td>Monensin</td>
<td>Ionophore</td>
<td>Cell membrane</td>
</tr>
<tr>
<td>Carbadox</td>
<td>Quinoxaline</td>
<td>DNA Synthesis</td>
</tr>
</tbody>
</table>

**Effect of STAT in Swine**

- Starter
- Grower
- Grower-Finisher

(Adapted from Zimmerman, J Animal Sci, 1986)

**Flowchart**

- **C57/B6**
  - STAT
  - Control
  - Measure properties
  - Analyze microbiome
  - Find relationships
Chronic, low-dose exposure: Body composition in STAT and control mice at age 10 weeks

Control – 22.9% body fat

STAT – 32.0% body fat

Control
Penicillin
Vancomycin
Pen + Vanc
Clortetracycline
All Antibiotics

*p<0.05

Ilseung Cho

FatSTAT: the effects of HFD and STAT on body composition

Laurie Cox
Cell 2014;158:705-21
FatSTAT: the effects of HFD and STAT on body composition

Male

Female

↑ High fat diet introduced    * p < 0.05 NC    ° p < 0.05 HFD
The effect of pre- or post-weaning antibiotic exposure

<table>
<thead>
<tr>
<th></th>
<th>Normal Chow</th>
<th>No antibiotics, 20 weeks</th>
<th>M</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursing</td>
<td>STAT-weaning</td>
<td>4 wks 16 weeks STAT penicillin</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>STAT-birth</td>
<td>20 weeks STAT penicillin</td>
<td></td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Body Composition at 20 weeks by DEXA scanning

* P < 0.05, ** p < 0.01, *** p <0.001

L Cox et al. *Cell* 2014;158:705-21
DuraSTAT: Are the changes **durable** with limited antibiotic exposure?

**Diet:**
- Nursing 0-4 weeks
- Chow 4-6w
- High Fat Diet 6-28 weeks

Control

4-STAT

8-STAT

28-STAT

Female C57

- Control n = 13
- 4-STAT n = 9
- 8-STAT n = 12
- 28-STAT n = 8

* P < 0.05, t-test

\( X_2 \): sacrificed 4 control and 4 8-STAT

\( X_3 \): sacrificed 3 control and 3 28-STAT
Effects of STAT on intestinal Th17 populations

8 week males

<table>
<thead>
<tr>
<th></th>
<th>Small intestine</th>
<th>Large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>STAT</td>
</tr>
<tr>
<td>IL-22</td>
<td>0.197</td>
<td>0.323</td>
</tr>
<tr>
<td>CD4+ production</td>
<td>96.4</td>
<td>3.07</td>
</tr>
<tr>
<td>IL-17A</td>
<td>0.206</td>
<td>0.269</td>
</tr>
<tr>
<td></td>
<td>97.5</td>
<td>2.01</td>
</tr>
<tr>
<td>IL17</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>IL22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p&lt;0.05 (t-test)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IL-17A

Small intestine

Large intestine

Jacqueline Leung
P’ng Loke Lab
Fecal community structure at 3 weeks

- **Control**
- **STAT**

Weeks: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28
Fecal community structure at 8 weeks

- **Control**
- **STAT**
- **Post 4-STAT**

Weeks: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28

Fat Mass (g)

Diet:
- **Milk**
- **NC**
- **HFD**

PC1 (9.2%)

PC2 (6.7%)

PC3 (4.8%)
Fecal community structure at 10 weeks
Fecal community structure at 28 weeks
28-week fecal community structure

- PC1 (9.2%)
- PC2 (6.7%)
- PC3 (4.8%)

- Control
- STAT
- Post 4-STAT
- Post 8-STAT
Changes in microbiome composition with limited STAT
Enriched taxa in the first 4 weeks of life

Across Experiments

→ in STAT
- *Lactobacillus* (3/3)
- *Allobaculum* (2/3)
- *Rikenellaceae* (2/3)

↑ in STAT
No findings consistent with other experiments
TranSTAT: is the growth phenotype transferable by microbiota alone?

**Donors** 18-week female C57BL/6J

**Recipients** 3-week female germ-free Swiss-Webster

Recipients housed for 35 days in specific pathogen-free (SPF) conditions
Is microbe-induced obesity transferable?

<table>
<thead>
<tr>
<th>Donors</th>
<th>Microbiota</th>
<th>Germ-Free Recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>No abx</td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
<td>5-weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No abx</td>
</tr>
</tbody>
</table>
Is microbe-induced obesity transferable?

Donors  | Microbiota  | Germ-Free Recipients
-------|-------------|----------------------
Control |             | No abx

Antibiotics

Body Composition - Days post-transfer

- Total
- Lean
- Fat

Control-conv. n = 7
STAT-conv. n = 8
Expression of genes involved in intestinal defenses in the microbiota donor and recipient mice

**Donors**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control-R</th>
<th>STAT-R</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROR T</td>
<td>0.0015</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>IL-17A</td>
<td>0.098</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>IL-17F</td>
<td>0.034</td>
<td>0.058</td>
<td></td>
</tr>
</tbody>
</table>

**Antimicrobial peptides**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control-R</th>
<th>STAT-R</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RegIII</td>
<td>0.055</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>b-Defensin</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p-values, by t-test
**HYPOTHESIS:** A series of short, therapeutic-dose pulses of antibiotic administered early in life will sufficiently change the gut microbiome to alter body composition.
Pat effects on alpha diversity

Antibiotic impact on long-term physiology through microbiota changes

STAT/PAT RESEARCH TEAM

Ilseung Cho
Laurie Cox
Shingo Yamanishi
Zhan Gao
Alex Alekseyenko
Barbara Methe
Yael Nobel
Victoria Ruiz
Isak Engstrand
Douglas Mahana

Statistics: Huilin Li
Mouse Care: Isabel Teitler, Minso Kim, John Hwang, Kartik Raju, Jiho Sohn, Jenn Chung
Immunology: Jackie Leung, P’ng Loke, Ken Cadwell
MRI: Sungheon Kim
Sequence Analysis: Erica Sodergren, George Weinstock, Sahar Abubucker, Makedonka Mitreva, Jiri Zavendil, Nicholas Bokulich, Tom Battaglia

Supported in by NIH 1UL1RR029893, T-R01DK090989, U54HG004968, U54HG003079, Diane Belfer Program for Human Microbial Ecology, Knapp Foundation, Ziff Foundation, HHMI
Early life microbiome disruption is associated with weight gain in humans

**Infant antibiotic exposures and early life body mass**
L Trasande, J Blustein, M Liu, E Corwin, LM Cox, MJ Blaser

**Association of caesarean delivery with child adiposity from age 6-weeks to 15 years**
J Blustein, T Attina, M Liu, AM Ryan, LM Cox, MJ Blaser, L Trasande

Exposed to antibiotics during the first 6 months of life
Diseases increasing in recent decades

Reflux Disease (GERD)

White men
Nonwhite men

Sources:
- *Ann NY Acad Sci* 2008 12:1150
- *Gut* 1997;41:594
New algorithm for child health

Analysis

Global microbes  Personal microbes

Administer

Assess for positivity, phenotypes
Human microbiome labmates at NYU