The "hygiene hypothesis"

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Acknowledgements
Quantitative RT-PCR
Allergy models
Geok Teng Seah
Laura Rosa Brunet, Jon Hunt
Claudia Zouany-Amorim
The impact of environmental variables on disease incidence

- Always diseased
- Disease now not in past
- Never diseased

Percent of population affected

Past

Present
You are **less** likely to be allergic if :-

- older siblings (boys > girls)
- rarely washed face and hands as a child
- brought up on a farm
- keep a dog
- lived in communist rather than western Europe
- dust in your home contaminated with bacteria
- infections via the faeco-oral route
- early BCG or TB or tuberculin positive (?)

You are **more** likely to be allergic if :-

- given antibiotics as a small child
How do we know that exposure to environmental bacteria has changed in the rich developed countries?

1. delayed hypersensitivity skin-test responses to saprophytic environmental mycobacteria have become rare

2. endotoxin levels are much higher in the bedding of farming than of non-farming families

3. endotoxin levels in the home correlate inversely with allergic sensitisation and allergic symptoms

4. different colonisation pattern in the neonate, and different and more rapidly changing adult gut flora in poor countries

von Mutius et al., Clin. Exp. Allergy. (2000); 30: 1230-1234
Geredia et al., Lancet (2000); 355: 1680-1683
Mammalian evolution took place in mud

More than 99% of *human* evolution has taken place in isolated hunter/gatherer communities

- soil, untreated water; mud
Evolution turns the inevitable into a necessity

- the environment in which you live must correspond to the genetically encoded knowledge
Why are mycobacteria important to the hygiene hypothesis?

1) Epidemiological links with reduced allergies
2) >80 species, ubiquitous, but different in different environments
3) Not normal commensal flora, so exposure determined by lifestyle
4) CD1-restricted T cells (long-lived mammals) that see only mycobacteria
5) Children without IFN$_\gamma$R or IL-12R die from mycobacterial infections.

6) Tuberculostearic acid in human lymph nodes
   Hanngren et al. Sarcoidosis (1987); 4: 101-104.
Exposure to endotoxin or other bacterial components might protect against the development of atopy

Endotoxin in bedding
EU/m²

<table>
<thead>
<tr>
<th>Group</th>
<th>Quantity (EU/m²)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farming families (n = 39)</td>
<td>49,479</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Nonfarming families with contact to livestock (n = 15)</td>
<td>23,340</td>
<td>P = 0.052</td>
</tr>
<tr>
<td>Controls (n = 30)</td>
<td>9,383</td>
<td>P = 0.121</td>
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</tbody>
</table>

Exposure to farming early in life and development of asthma and allergy: a cross-sectional survey

Adapted from Riedler et al., Lancet (2001) 358: 1129-1133

<table>
<thead>
<tr>
<th></th>
<th>Stables and farm milk in the 1st year of life (n=218)</th>
<th>Neither stables nor farm milk exposure (n=170)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma diagnosis</td>
<td>1% (3)</td>
<td>12% (20)</td>
</tr>
<tr>
<td>At least one wheeze attack in past 12 months</td>
<td>3% (6)</td>
<td>15% (25)</td>
</tr>
<tr>
<td>Hay fever</td>
<td>3% (7)</td>
<td>16% (27)</td>
</tr>
<tr>
<td>Runny nose and itchy eyes in past 12 months</td>
<td>5% (11)</td>
<td>20% (34)</td>
</tr>
<tr>
<td>Atopic sensitisation</td>
<td>12% (27)</td>
<td>33% (56)</td>
</tr>
</tbody>
</table>
Two major types of effector T lymphocyte

- **Th0 (non-activated)**
- **Th1 lymphocyte**
  - Attacks tuberculosis, other bacteria, cancer, many viruses
- **Th2 lymphocyte**
  - Allergies
  - Immediate explosive reactions to parasites
Variation in background cytokine expression, even in fresh UNSTIMULATED peripheral blood mononuclear cells from tuberculin-positive donors with no allergies.
Relation between the occurrence of Type 1 diabetes and asthma

More than 4 episodes of wheeze in past 12 months (%)

In incidence of type 1 diabetes per 100000 person years

The incidence of inflammatory bowel disease is increasing.

### Diseases of "immunodysregulation"; three groups

<table>
<thead>
<tr>
<th>Disease group</th>
<th>Pathological target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergies</td>
<td>aeroallergens</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>gut contents</td>
</tr>
<tr>
<td>Autoimmunity</td>
<td>self</td>
</tr>
</tbody>
</table>
Most of the time the immune system must make the decision NOT to mount an immune response, despite the existence of lymphocytes that recognise the molecule being scanned.

Inputs to the immune system:
- Continuous sampling of self
- Input from the environment

Do NOT attack:
- Self
- Gut contents
  - 1.5 kg of bacteria
  - Dinner, wine etc.
- Harmless allergens in air
  - Pollen
  - House dust mite

ATTACK:
- Infection
- Mutation (possibly cancer)
  - "Danger"

Regulatory cells

Effector cells (Th1 or Th2)
The “hygiene hypothesis” is explained by an “Effector/Regulator” balance, not by a Th1/Th2 balance.

(Th1) 

(Th2) 

(IL-4, NO IL-10) 

(T_{reg}) 

(IL-10, ± IL-4)
Children with type-1 diabetes and their unaffected siblings have fewer symptoms of asthma

Douek et al., Lancet (1999) 353:1850
The “hygiene hypothesis” is explained by an “Effector/Regulator” balance, not by a Th1/Th2 balance.

Why do we need microbial exposure to prime these cells?
## Regulatory lymphocytes

Many can secrete IL-4, but they must be distinguished from Th2 effector cells

<table>
<thead>
<tr>
<th></th>
<th>Secrete: IL-4</th>
<th>IL-10</th>
<th>Evoked by bacteria</th>
<th>Allergy</th>
<th>Regulate: Autoimmunity</th>
<th>IBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tr1 Th3 CD25+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Anergic</td>
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<tr>
<td>CD30+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>B7.2+</td>
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<tr>
<td>CD8+ γ/δ</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>CD4+ γ/δ</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>CD8+ α/β</td>
<td>+</td>
<td>IL-4 or IL-482</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>NKT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>+</td>
<td>+</td>
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</table>
Regulatory networks mature in the periphery in the presence of "self" and microbial antigens.

**THYMUS**

- T cell repertoire selected in thymus; → weakly anti-self.
- Regulatory T cell precursors

**PERIPHERY**

- Regulatory network maturation
- APC
- Self version
- Microbial version + "danger signals"
- Bacteria

GUT ?
What is the role of microbial components in the induction of regulatory T cells?

- Need microbial homologues of conserved proteins, presented in the context of “danger signals”, for comparison with self? - hsp

- Need microbial components that modulate APC function
  - cause APC to secrete IL-10 rather than IL-12?
  - downregulate Toll-like receptors, upregulate Notch/Notch ligand?
  
  → act as $T_{reg}$ adjuvant - *M. vaccae*

- Can’t induce oral tolerance in germ-free mice

- ? induction of $T_{reg}$ requires a Th1 cytokine background (IFN-gamma)
Can we prove that vaccines non-specifically modulate disease?

1. Bacterial vaccines can treat immunoregulatory disorders in mice

2. Encouraging clinical trials using bacterial vaccines to treat human immunoregulatory disorders

3. Epidemiological studies reveal non-specific effects of human vaccinations
Mycobacterium vaccae s.c. or orally, or some of its components s.c., induce spleen cells that secrete IL-10 in response to ovalbumin.
Treatment with a mycobacterial preparation can enable development of allergen-specific, IL-10-dependent, CD4+ regulatory T cells

-21  0  12  21  22
Mycobacterial vaccine (SRP299) or saline s.c.  ovalbumin/alum i.p.  PBS or ovalbumin aerosol  Transfer CD4+ T cells +/- anti-IL-10

0  12  21  23
ovalbumin/alum i.p.  PBS or ovalbumin aerosol  BAL  BAL
Susceptibility to induction of autoimmune arthritis is affected by the nature of the bowel flora

Germ-free animals derived by caesarian section

Reconstitute intestinal flora with different bacterial species

<table>
<thead>
<tr>
<th>Species A</th>
<th>Susceptibility to induction of arthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very susceptible</td>
<td></td>
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</table>

<table>
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<tr>
<th>Species B</th>
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<tbody>
<tr>
<td>Resistant</td>
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</table>

Normal rat

Susceptible

Placebo-controlled study of SRL172 (derived from heat-killed Mycobacterium vaccae) in the treatment of hayfever

Prof. Julian Hopkin

3 doses of SRL172 monthly intervals

Diary cards & immunology weeks 10-20

Mar  Apr  May  Jun  Jul

SUBMITTED FOR PUBLICATION ELSEWHERE

ABSTRACT

Skin surface area affected by atopic dermatitis and dermatitis score, before and after treatment

Arkwright and David, J Allergy Clin Immunol (2001) 107:531-4

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>SRL 172</th>
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<tbody>
<tr>
<td><strong>Skin area affected</strong></td>
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<tr>
<td>(Mean % ±SD)</td>
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<tr>
<td>Before treatment</td>
<td>40 ± 30</td>
<td>38 ± 30</td>
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<tr>
<td>1 month after treatment</td>
<td>37 ± 29</td>
<td>30 ± 31</td>
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<tr>
<td>3 months after treatment</td>
<td>40 ± 33</td>
<td>21 ± 24</td>
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<tr>
<td><strong>Dermatitis score (0-300)</strong></td>
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<tr>
<td>(median (interquartile range))</td>
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<tr>
<td>Before treatment</td>
<td>38 (22-86)</td>
<td>55 (19-69)</td>
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<tr>
<td>1 month after treatment</td>
<td>40 (22-76)</td>
<td>22 (8-74)</td>
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<tr>
<td>3 months after treatment</td>
<td>28 (14-71)</td>
<td>14 (6-41)</td>
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</table>
Adjuvant therapy with BCG vaccine in relapsing-remitting multiple sclerosis


1) Gadolinium-enhanced MRI scans, monthly, 6 m

2) A single dose of BCG vaccine i.d.

3) Repeat MRI scans, monthly, 6 m

51% reduction in Gd-enhancing, 57% reduction in active lesions, p=0.008 Wilcoxon signed rank test
Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa


Survival probability

Follow up (months)

BCG only (n=717)
BCG + DTP (n=2164)
No vaccine (n=1091)
Has the childhood vaccination schedule taken over from the microbial environment as “educator” of the immune system?

- Environmental micro-organisms, in accordance with mammalian evolutionary history
- VACCINES
- Antibiotics
  - Hygiene

Correctly primed immunoregulation

Incorrectly primed immunoregulation
- ??? Increasing
  - allergies
  - autoimmunity
  - IBD
Does this immunisation schedule correctly prime immunoregulatory mechanisms.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Birth</th>
<th>1 mo</th>
<th>2 mos</th>
<th>4 mos</th>
<th>6 mos</th>
<th>12 mos</th>
<th>15 mos</th>
<th>18 mos</th>
<th>4–6 yrs</th>
<th>11–12 yrs</th>
<th>14–16 yrs</th>
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<tr>
<td>Hepatitis B†</td>
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<td>Diphtheria and tetanus toxoids</td>
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<td>and pertussis</td>
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<tr>
<td><em>H. influenzae</em> type b†</td>
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<td>Poliovirus**</td>
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<td>Rotavirus**</td>
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<tr>
<td>Measles-mumps-rubella#</td>
<td>Rv</td>
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<tr>
<td>Varicella†</td>
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http://www.cdc.gov/mmwr/preview/mmwrhtml/00056261.htm
Conclusions

Hygiene, antibiotics and vaccines are the three most beneficial achievements of medical research.

Epidemiological, theoretical, evolutionary, experimental and clinical trial evidence suggests that altered priming of the immune system can lead to deficient IMMUNOREGREULATION

Deficient IMMUNOREGREULATION might contribute to increasing
- allergies
- autoimmune disease
- inflammatory bowel disease
Conclusions

The solution

- better understanding of immunoregulation

- vaccines to drive immunoregulatory networks
  (e.g. no specific target infection)

- vaccines that replace rather than prevent infection

- vaccines that treat diseases of immunodysregulation

Hygiene, antibiotics and vaccines are the three most beneficial achievements of medical research.