Centre d'immunologie et des Maladies Infectieuses

5V569 - Atelier de allergie

Microbiota and Allergy

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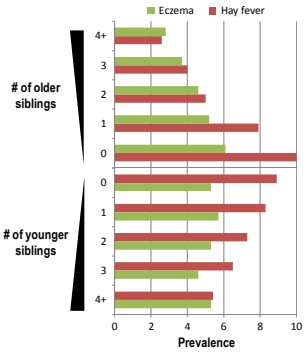
Outline

1. Hygiene theory
2. Epidemics of allergy – environmental factors
3. The gut microbiota and our digestive system
4. Gut microbiota and host immunity
5. Antibody responses
6. Model of allergy mechanisms
7. Gut microbiota and its role in disease
8. Gut microbiota in early life
9. Self-non-self versus the danger model.
10. Gut microbiota and allergy
11. Solutions

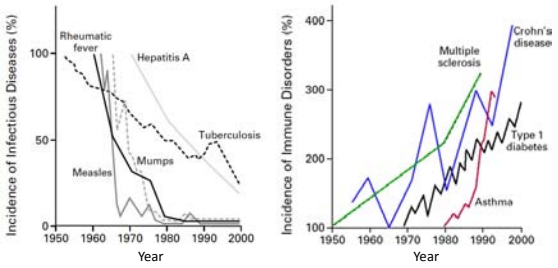
Birth of the hygiene theory - by Pr. David P Strachan

- Prevalence of hay fever and eczema depends on position in household.
- **Older siblings** are protective of allergy
- **Younger siblings** have small effect on hay fever and no effect on eczema.
- **Hypothesis:** Exposure to infectious events through unhygienic contact with siblings protect from allergy.



Strachan et al. BMJ 1989

Hygiene theory and chronic inflammatory diseases

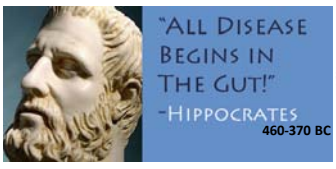


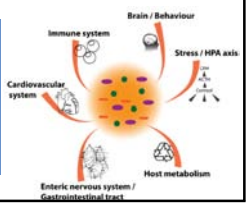
Disappearance of prototypic infectious diseases inversely correlate with occurrence of chronic inflammatory diseases.

Bach *et al.* NEJM 2002

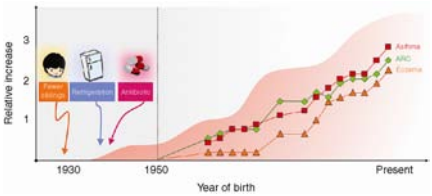
Hygiene theory and allergic diseases

- Massive increase in prevalence of allergic diseases in Westernized countries (>20% over 10 year period)
- Allergic disease is attributed to both genetic predisposition and environmental factors
- Genetic drift over such a short period of time cannot explain increased incidence of disease
- Westernized life-style has introduced several environmental risk factors that disturb the homeostatic balance of gut microbiota





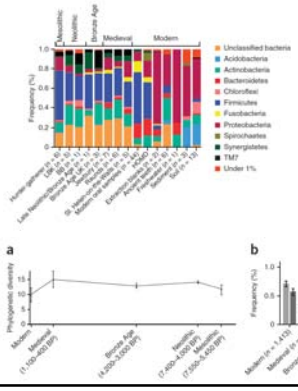
Lifestyle changes affecting Gut Microbiota



- Westernized life-style has introduced several environmental risk factors that disturb the homeostatic balance of gut microbiota
- Excessive antibiotic use, especially during early life (or even during pregnancy)
- Shift towards more formula-fed babies
- Shift towards greater numbers of babies born by Caesarean section
- Western diet

Abrahamsson *et al.* JACI 2015

Oral microbiota shifts through history of mankind

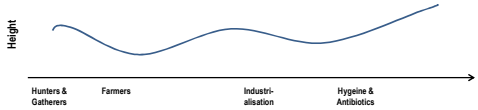


Two of the greatest dietary shifts in human evolution involved :

- Adoption of carbohydrate-rich Neolithic (farming) diets (beginning ~10,000 years before the present)
  - P. gingivalis augments.
- Industrially processed flour and sugar (in ~1850)
  - Streptococcus mutans augments (associated with carries).
- Reduced oral microbiota diversity in the modern human.

Adler *et al.* Nat Genetics 2013

Microbial transmission and population height



Hypothesis: Microbial transmission regulates population height

Urbanization and dense populations are associated with microbial transmission. Population height increases with low microbial transmission.

The last siblings are often shorter than the first siblings in child-rich families.

Height is associated with socio-economic status.

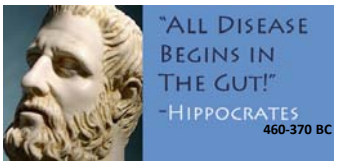
Adopted children before age 5 become significantly higher than the average of the native country and approaches the height of the adoption country.

Clearly genetics cannot explain the rapid height increase (1cm/decade in the 20th century Scandinavia and USA) with abrupt drop for children growing up during wartime.

However, it is difficult to separate a better diet and changes in the gut microbiota with microbial transmission.

Beard & Blaser Perspect Bio Med 2002

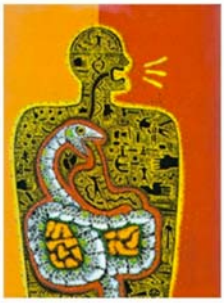
Dysbiosis - cause or consequence - and so what?



"Le malade imaginaire"
Molière, 1673

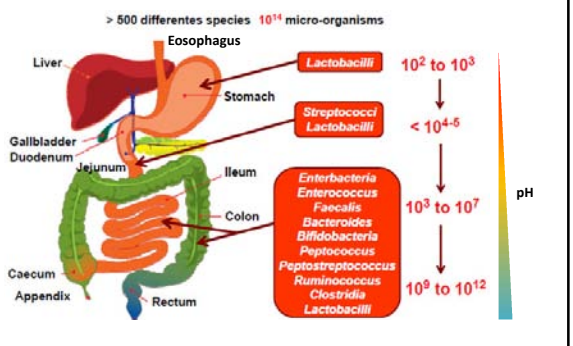
- Prescription of "lavement" for:
  - Digestion, intestinal secretion and bad mood.
- Dates back at least to 100 B.C.

The human Gut and its inhabitants in numbers



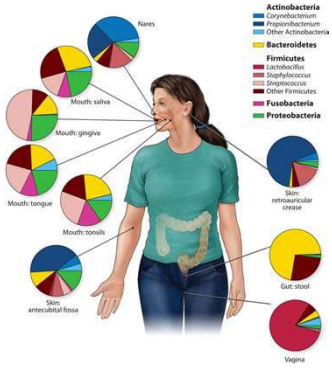
- 30 tons of food and 50.000 L during a lifetime
- Huge mucosal surface:150-200 m^2
- >50 billions of new bacteria every day
- 70-80% of all immune cells are located in the Gut.
- 1-2g secretory IgA per day
- 100 millions of neurons (as many as in the spinal cord).
- 10^{14} bacteria: x10 number of cells in the entire body, i.e. 1-2 kg.
- 100 times more bacterial genes than human genes.

Spatial distribution of gut microbiota



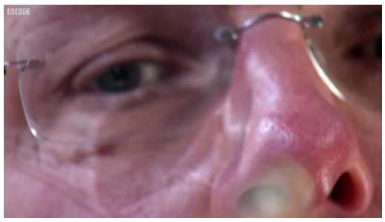
http://www.jpp.krakow.pl/journal/archive/08_15/articles/02_article.html

Human body sites harbor distinct microbiota



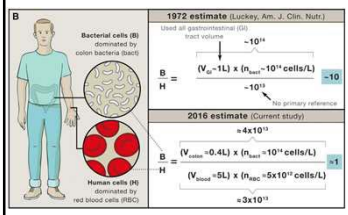
Grice *et al.* Ann Rev Gen Hum Genet 2012

The digestive system - A trip through the GI tract

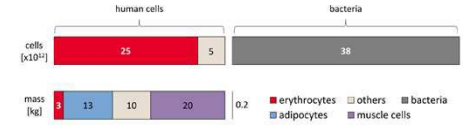


- Mouth (cheewing + saliva containing enzymes)
- Eosophage
- Stomach (very acidic, proteases, few microbes)
- Small intestine (somewhat acidic, microbes (degrade otherwise none-degradable plant fibers).
- Large intestine (Final digestion and return of water to host)
- You all know what happens then.....**COLLECTION TIME!**

We are outnumbered and outsmarted



Population	Bacteria:Human
Man	1.3
Woman	2.2
Young Infant	2.3
Infant	1.7
Elder	1.8
Obese	1.4

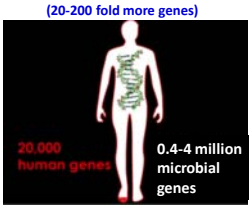


Of note, only takes into account the colon, but demonstrate that the colon contains 98% of all our commensal bacteria.

Sender et al. Cell 2016 & Sender et al. PLoS Biol 2016

We are outnumbered and outsmarted

Outsmarted
(20-200 fold more genes)

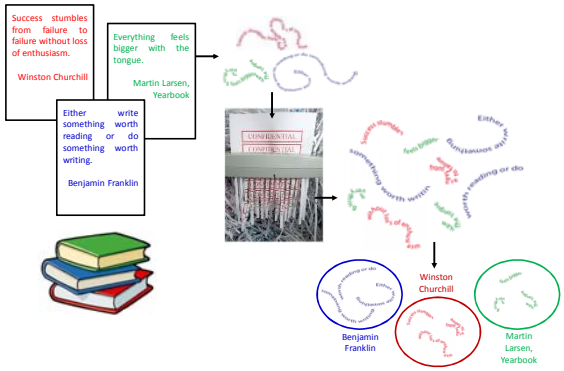


Each bacterial species has its own genomic DNA (all human cells have identical genomic DNA).

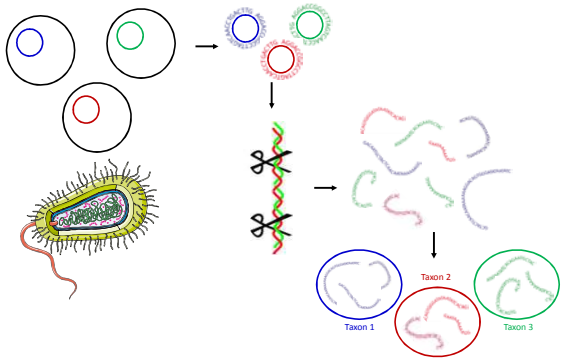
Bacterial genome (2-10x10^6 bps) versus human genome (3x10^9 bps)

Bacterial DNA is much more compact than human DNA in terms of gene content.

Next Generation Sequencing - like reading a threaded book



Next Generation Sequencing - like reading a threaded book



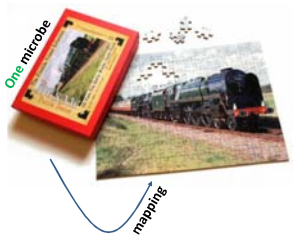
Microbial whole genome sequencing

?assembly

- One microbe is fully sequenced and assembled.

One microbial genome puzzle

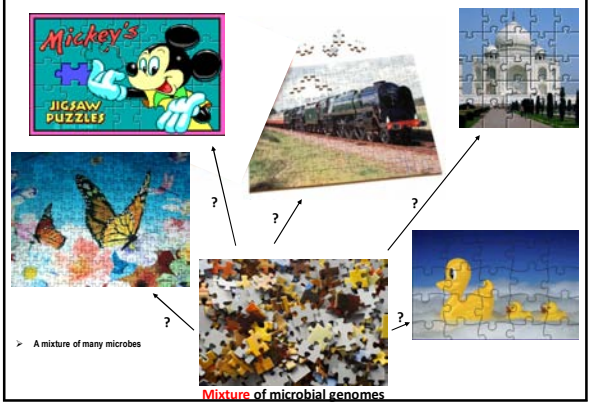
Microbial whole genome sequencing



- Using the reference lid jigsaw genes derived from the reference may be identified.

One microbial genome puzzle

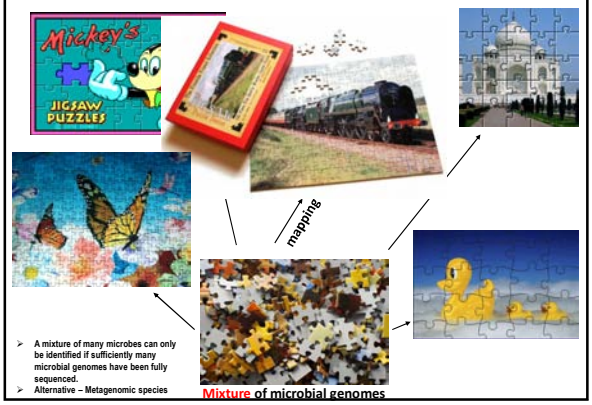
Metagenomic sequencing



- A mixture of many microbes

Mixture of microbial genomes

Metagenomic sequencing

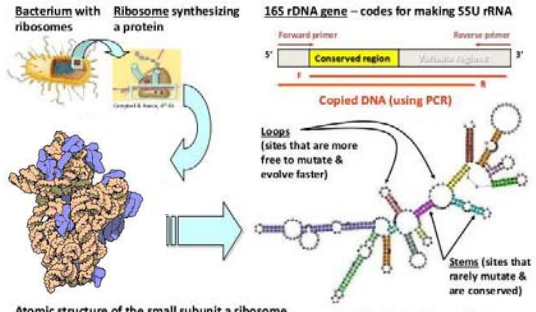


- A mixture of many microbes can only be identified if sufficiently many microbial genomes have been fully sequenced.
- Alternative – Metagenomic species

Mixture of microbial genomes

16S rRNA sequencing

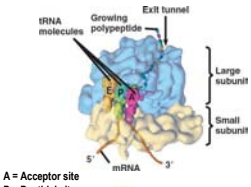
Use of primers to copy the 16S rDNA gene in bacteria



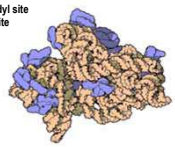
Atomic structure of the small subunit a ribosome. The rRNA, shown in orange, helps match the mRNA (codon) to the tRNA (anticodon).

Small subunit ribosomal RNA

16S rRNA sequencing

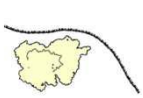


A = Acceptor site
P = Peptidyl site
E = Exit site

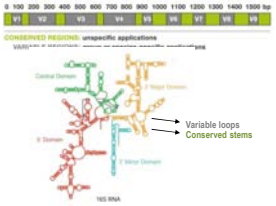


- 16S rRNA in orange
- Associated protein subunits in blue

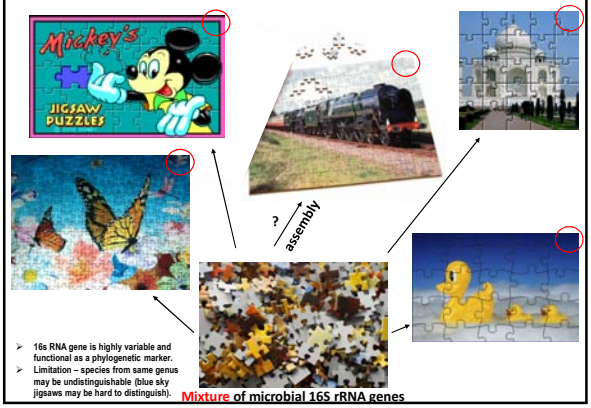
Protein synthesis in the
1. Cytosol
2. Endoplasmic reticulum



16S rRNA primary and secondary structures



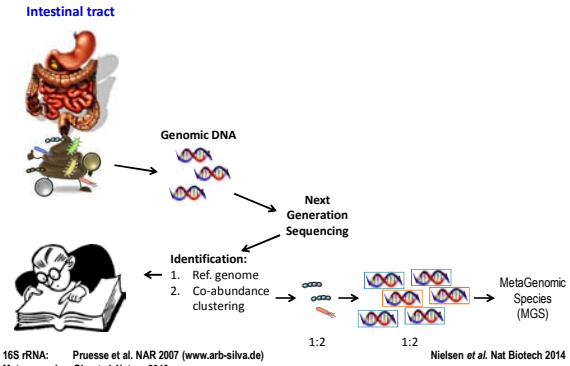
16S rRNA sequencing



- 16s RNA gene is highly variable and functional as a phylogenetic marker.
- Limitation – species from same genus may be undistinguishable (blue sky jigsaws may be hard to distinguish).

Mixture of microbial 16S rRNA genes

Big data in Microbiology



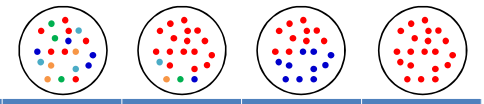
16S rRNA: Pruesse et al. NAR 2007 (www.arb-silva.de)
Metagenomics: Qin *et al.* Nature 2010
Nielsen *et al.* Nat Biotech 2014

Biodiversity

Quantitative
Biomass (g)

- A community's biodiversity correlates with it's size and location
- Ecologists measure biodiversity as heterogeneity, which considers both diversity factors: richness and relative abundance.
- $H = -\sum_{i=1}^{k} p_i log(p_i)$; p=abundance
- $E_H = \frac{H}{log(k)}$; [Normalized]

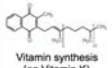
Qualitative
Richness
Diversity



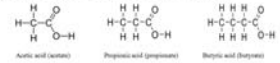
Species richness	4	4	2	1
Shannon entropy [normalized]	p_i=1/5 H=log(5) [E_H=1]	p_{red} ~ 1| $p_{NOT\ red}$ ~ 0 H= ~0 [E_H= ~0]	p_i=1/2 H=log(2) [E_H=1]	p_i=1 H=0 [E_H=0]

The many roles of gut Microbiota

- Increases the metabolic capacity of the host.
  - Digestion of otherwise unused food components.

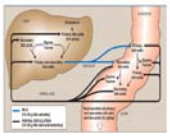


Vitamin synthesis (eg Vitamin K)



Production of short chain fatty acids

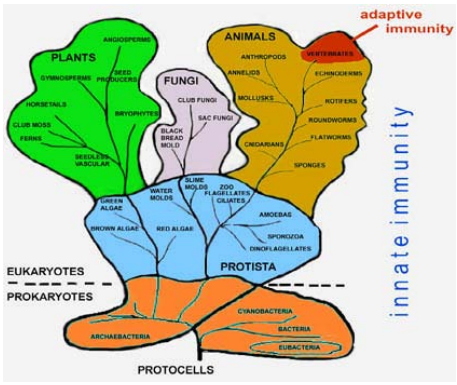
Completion of the bile-salt cycle
Lipid uptake



Protect the host from colonization with pathogenic bacteria (Colonization resistance)

Immunity

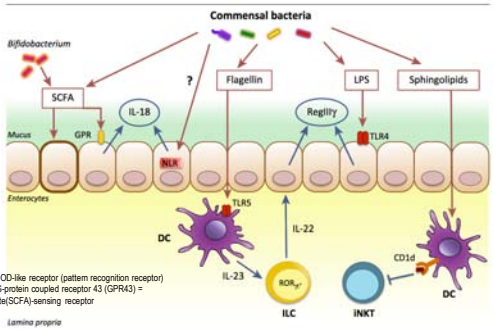
Evolution of Adaptive immunity



http://bio1510.biology.gatech.edu/module-5-integrative-health/03-adaptive-immunity

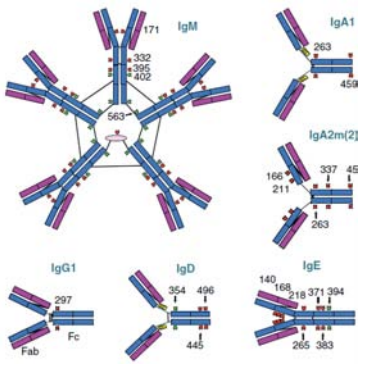
Gut Microbiota in health - Innate immunity

- Microbiota influences intestinal immune responses primarily through the expression of pathogen-associated molecular patterns (PAMPs) and metabolic by-products.



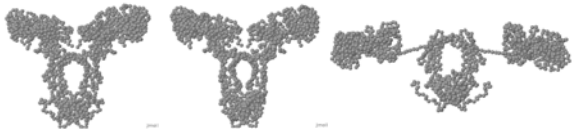
NLR = NOD-like receptor (pattern recognition receptor)
GPR = G-protein coupled receptor 43 (GPR43) = metabolite(SCFA)-sensing receptor

Antibody characteristics



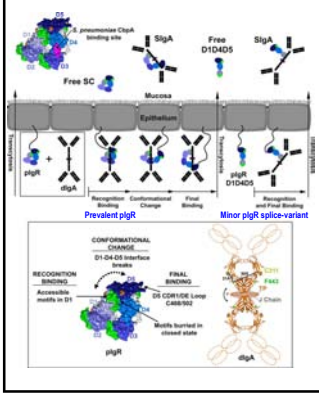
Arnold et al Ann Rev Immunol 2007

Antibody characteristics



IgG	IgA2	IgA1
Small mammals Higher monkeys	Small mammals Higher monkeys	**Not found in** Small mammals Higher monkeys
Y-shape Sensitive to proteases	Y-shape Non-Sensitive to proteases	T-shape Less-Sensitive to proteases
Non-Secretory	Secretory : non-planar (fab fragment not aligned with Fc portion) Binds to Peyer's Patch M cells to undergo transcytosis thereby delivering antigens to GALT Mantis NJ *et al.* JI 2002	Secretory : planar (fab fragments aligned with Fc portion No binding to Peyer's Patch M cells. Warning! Assay used mouse M cells. Rochereau N *et al.* PLoS Biol 2013

Poly Ig Receptor (pIgR), Secretory component (SC) and pIgs

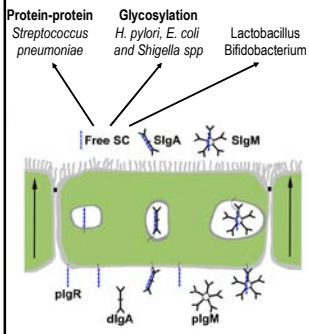


Human pIgR:
- Glycosylated type I membrane protein
- Five tandem immunoglobulin-like (Ig-like) domains (620AA)
- Transmembrane domain (23AA)
- Intracellular domain (103AA)

pIgR in evolution
- Oldest identifiable Fc receptor,
- Teleost (bony) fish (2 Ig-like domains)
- Birds, amphibians and reptiles (4 Ig-like domains)
- Mammals (5 Ig-like domains (D1-D5)) plus a splice variant (D1D4D5)

Stadtmueller et al. eLife 2016

Poly Ig Receptor (pIgR), Secretory component (SC) and pIgs



Secretory component
- <50% of pIgR transcytose in empty state.
- Free SC may bind bacteria and toxins.
  - *Streptococcus pneumoniae* (protein-protein)
  - *H. pylori, E. coli* and *Shigella spp* (glycosylation)
  - Lactobacillus and Bifidobacterium (unknown binding mode)
- Free SC may bind mucus (glycosylation dependent).
- Free SC binds pIgM 10x stronger than dIgA (maybe not identical to pIgR affinity)

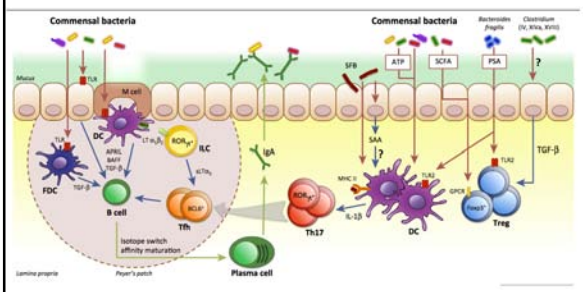
pIgR – dimeric-IgA (dIgA)
- Bound by pIgR of all tetrapods (J-chain is equally conserved).
- Bound by D1 followed by D5 (D5 is finally covalently attached)

pIgR – pentameric-IgM
- Bound by D1 but not D5.
- Not covalently linked

Brandtzaug et al. Immunochemistry 1977, Stadtmueller et al. eLife 2016

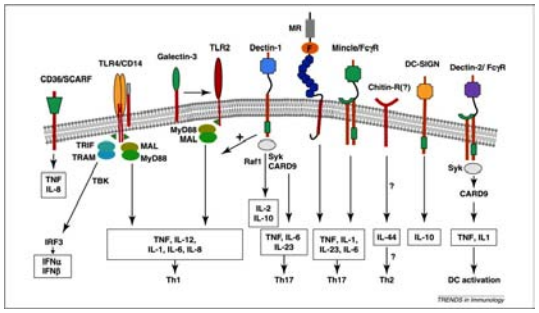
Gut Microbiota in health - adaptive immunity

- Microbiota stimulation leads to B cell switch to IgA, regulatory T cell induction, T cell differentiation to Th17



SAA=Serum amyloid A; GPCR=G protein-coupled receptor; PSA=Polysaccharide A; ILC=Innate lymphoid cells

Microbiota, Toll-like receptors and T cell differentiation

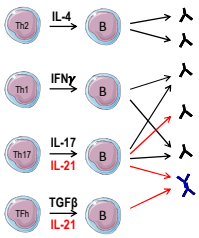


- House Dust Mite derived chitin induce IgE responses via a TLR2 dependent pathway (independent of TLR4 and 6)

Choi *et al.* All Asthma Immunol Res 2016

Netea *et al* Trends Immunol 2010

Orchestration of antibody class-switching

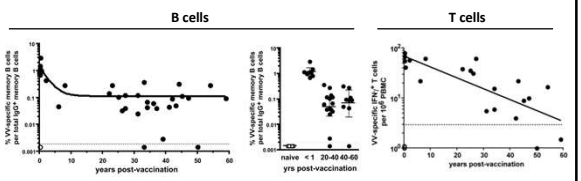


Mouse	Human	Role
IgE	IgE	Bind mast cells
IgG1	IgG4	Bind mast cells
IgG2a	IgG1	Fix complement, bind protein
IgG2b	IgG3	Fix complement, bind protein
IgG3	IgG2	Recognize carbohydrate epitopes
IgA	IgA1/2	Mucosal antibody

- Mouse and Human antibody class-switching is coordinated by T cell help.

Dullaers *et al.* Immunity 2008; Mitsdoerffer *et al.* PNAS 2010; Hussain *et al* Clin Diag Lab Immunol 1995

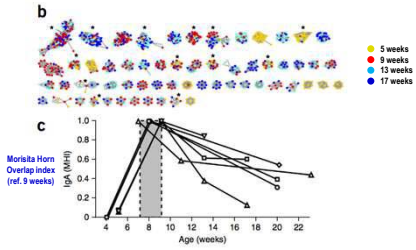
Temporal changes of antibody immunity



- B cell retraction phase is followed by long-term (+50 years) stable maintenance of B cell memory.
- T cells continue to retract, but remain detectable for more than 50 years.

Crotty *et al.* JI 2003

Temporal changes of antibody immunity



- 5 weeks CDR3 sequences cluster apart from the rest (yellow clusters), but from 9 weeks forward mice tend to have persisting CDR3 sequences in their IgA B cell repertoire (multi-color mixed clusters).
- The Morisita-Horn Overlap Index (MHI) equally shows more overlap between 9 weeks and later time points compared with earlier time points.

Lindner *et al.* Nat Immunol 2015

Allergy - Mechanism recall

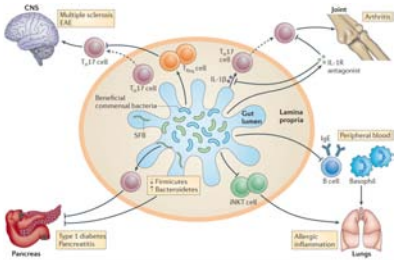
- Simplified mechanism:
  - 1st exposure: **A.** Allergen presenting APCs prime T cells. **B.** B cells bind allergens, class switch (IgE) and affinity mature with the help of T cells. **C.** B cells secrete IgE which is bound by mast cells.
  - 2nd exposure: **A.** Allergen cross-link IgE on Mast cells provoking degranulation and thus release of histamine and prostaglandins. **B.** Vasodilation and mucus secretion.

Mechanism in action - Asthma

- Simplified mechanism:
  - 1st exposure: **A.** Allergen presenting APCs prime T cells. **B.** B cells bind allergens, class switch (IgE) and affinity mature with the help of T cells. **C.** B cells secrete IgE which is bound by mast cells.
  - 2nd exposure: **A.** Allergen cross-link IgE on Mast cells provoking degranulation and thus release of histamine and prostaglandins. **B.** Vasodilation and mucus secretion.

Gut microbiota and autoimmunity

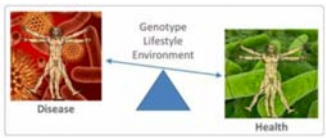
Autoimmunity associated with gut microbiota

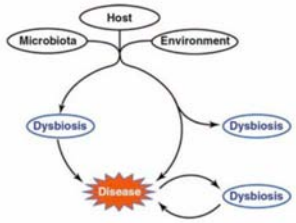


- IBD (Arumugam *et al.* Nature 2011, Juste *et al.* Gut 2014, Palm *et al.* Cell 2014)
- Type-1 Diabetes (Qin *et al.* Nature 2012, Markle *et al.* Science 2013)
- Arthritis (Scher *et al.* Nat Rev Rheumatol 2011, Scher *et al.* eLife 2013)
- Allergy (Russell *et al.* EMBO Rep 2012)
- **EAE / Multiple sclerosis** (Berer *et al.* Nature 2011, Miyake *et al.* PLoS One 2015)

Kamada *et al.* Nat Immunol Rev 2013

Dysbiosis - cause or consequence of disease?





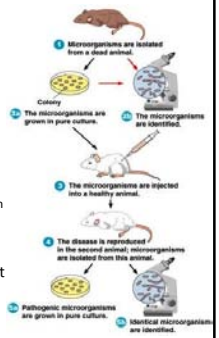
- Genetic or environmental factors may lead to dysbiosis
- Dysbiosis may lead to disease
- Genetic or environmental factors may lead to disease irrespective of dysbiosis.
- Disease may lead to dysbiosis

Koch's postulate and why it doesn't always apply

Koch's Postulates
(Robert Koch and Friedrich Loeffler in 1884)

Evidence required to establish etiologic relationship between microorganism and disease:

- Microorganism must be observed in every case of the disease
  Criticism: Healthy carriers exist (Cholera, Thypoid, but also viruses like Zooster and HIV)
- It must be isolated and grown in pure culture
  Criticism: Not all microbes can be cultivated and viruses only in presence of their host. Effective vaccines eradicating e.g. polio is considered a good proof of the causality of polio virus.
- The pure culture, when inoculated in animals, must reproduce the disease
- Microorganism must be recovered from the diseased animal.



Bradford Hill criteria - epidemiological alternative to Koch

Bradford Hill Criteria
(Epidemiologist Sir Austin Bradford Hill in 1965)

- **Strength (effect size):** A small association does not mean that there is not a causal effect, though the larger the association, the more likely that it is causal.
- **Consistency (reproducibility):** Consistent findings observed by different persons in different places with different samples strengthens the likelihood of an effect.
- **Specificity:** Causation is likely if there is a very specific population at a specific site and disease with no other likely explanation. The more specific an association between a factor and an effect is, the bigger the probability of a causal relationship.
- **Temporality:** The effect has to occur after the cause (and if there is an expected delay between the cause and expected effect, then the effect must occur after that delay).
- **Biological gradient:** Greater exposure should generally lead to greater incidence of the effect. However, in some cases, the mere presence of the factor can trigger the effect. In other cases, an inverse proportion is observed: greater exposure leads to lower incidence.
- **Plausibility:** A plausible mechanism between cause and effect is helpful (but Hill noted that knowledge of the mechanism is limited by current knowledge).
- **Coherence:** Coherence between epidemiological and laboratory findings increases the likelihood of an effect. However, Hill noted that "... lack of such [laboratory] evidence cannot nullify the epidemiological effect on associations".
- **Experiment:** "Occasionally it is possible to appeal to experimental evidence".
- **Analogy:** The effect of similar factors may be considered.

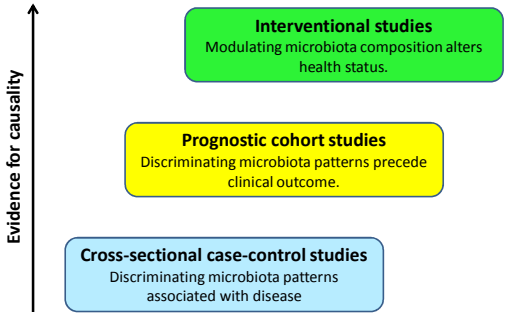
Dysbiosis - cause or consequence - and so what?

"Le malade imaginaire"
Molière, 1673

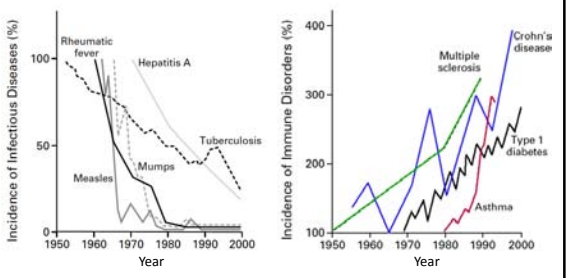
- Prescription of "lavement" for:
  - Digestion, intestinal secretion and bad mood.
- Dates back at least to 100 B.C.

- The bi-directional interactions between gut microbiota, metabolic and endocrine functions of the organism suggest that impacting one will impact the other.
- If the gut microbiota is not the cause:
  - Treatment targeting the microbiota will not be curative,
  - but may temporarily cure symptoms.
  - Many treatments actively used are non-curative (e.g. HIV therapy)
- If the gut microbiota is the cause:
  - Treatment targeting the microbiota is curative (*Clostridium difficile* infections),

Study design defines the ability to determine causality



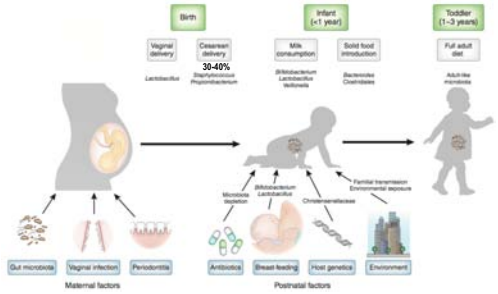
Hygiene theory and autoimmunity



Disaperence of prototypic infectious diseases inversely correlate with occurrence of autoimmune disease.

Bach *et al.* NEJM 2002

Early-life factors affecting infant gut microbiota

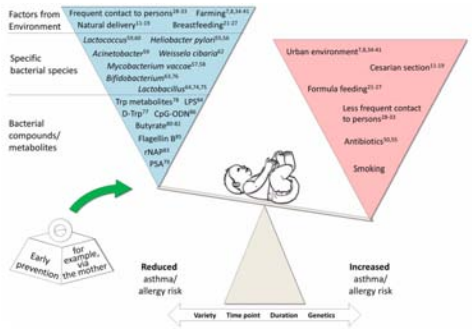


- Both maternal and environmental factors influence early-life gut microbiota colonization.

Tamburini *et al.* Nat Med 2016

C-section and allergy

C-section and allergy



Jatzlauk *et al.* Allergy 2017

C-section and allergy

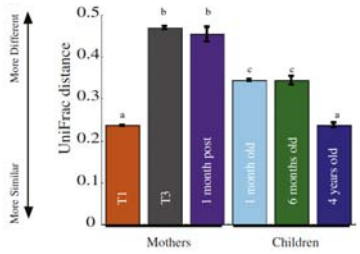
- **Obesity:** Caesarean born babies are at double the risk of becoming obese.
- **Allergy:** Associated with elective/planned C-section (odds ratio=1.49 [1.13-1.97]). Not significant for emergency C-sections (n>60.000).
  - An intact amniotic sac (more frequent in elective C-section) is associated with allergy. Breaking the amniotic sac may result in the first bacterial exposure.

  Sevelsted *et al.* J Pediat 2016; Rusconi *et al.* Am J Epid 2017
- **Asthma:** Elective C-section (OR = 1.58 [1.17-2.13], n=1400).
  - Exclusive Breastfeeding for 6 months (OR = 1.39 [0.92-2.10]).
  - Non-exclusive breastfeeding or bottle feeding (OR = 1.91 [1.22-2.99]).

  Chu *et al.* PLoS One 2017
- **Gut colonization** at 1 week:
  - C-section: Citrobacter freundii, Clostridium species, Enterobacter cloacae, Enterococcus faecalis, Klebsiella oxytoca, Klebsiella pneumoniae, and Staphylococcus aureus
  - Vaginal: Escherichia coli
  - Differences disappear before Age 1.
  - Initial airway microbiota was unaffected by birth method.

  Stockholm et al. JACI 2016

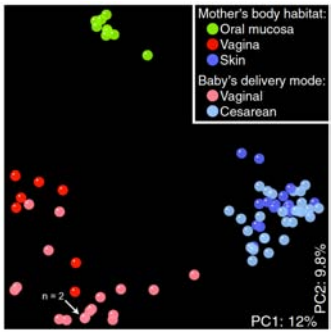
Microbiota alterations during pregnancy and early life

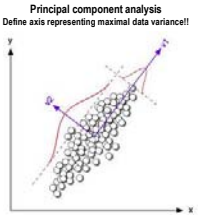


- Maternal microbiota changes between 1st and 3rd trimester and remains altered until at least 1 month post birth.
- Infant microbiota is different from initial T1 maternal gut microbiota.
- Children approaches the maternal microbiota at 4 years of age.

Koren *et al.* Cell 2012

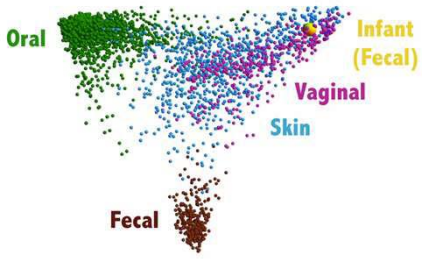
Delivery mode shape early life gut microbiote colonization





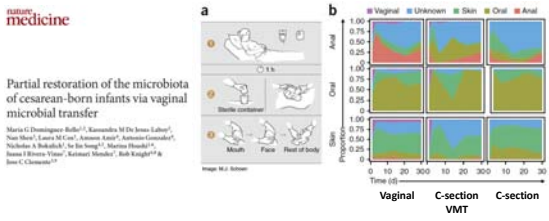
Dominguez-Bello *et al* PNAS 2010

Gut microbiota maturation during first 2 years of life



Dr. Rob Knight

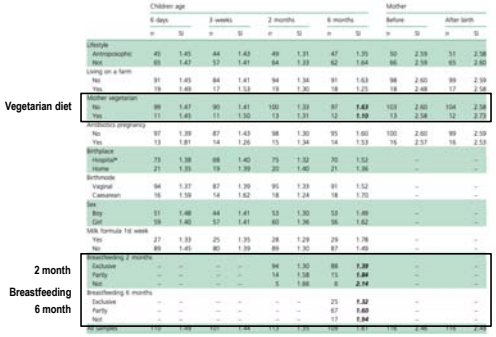
Vaginal Microbial Transfer (VMT) rescue microbiota post c-section.



- Vaginal Microbial Transfer (VMT) partly rescue gut microbiota composition of children born by C-section.
- Would primarily be maternal vaginal microbiota (mother/baby paired).
- However, in the case of mothers treated with antibiotics (such as HIV infected mothers) allogenic microbiota may be of interest – even for children born vaginally.

Dominguez-Bello *et al.* Nature Medicine 2015

Early-life factors affecting infant gut microbiota



- **Birth route:** C-section associated with increased diversity in early-life
- **Breastfeeding:** Microbiota diversity reduced at 6 months.
- **Mother diet:** Vegetarians => reduced diversity at 6 months

Hesla *et al.* FEMS Microb Ecol 2014

Early-life factors affecting infant gut microbiota

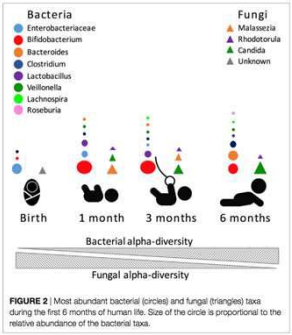
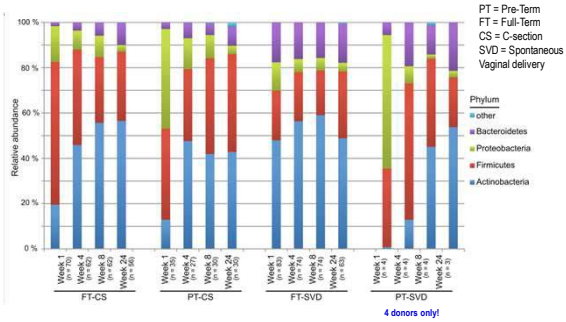


FIGURE 2 | Most abundant bacterial (circles) and fungal (triangles) taxa during the first 6 months of human life. Size of the circle is proportional to the relative abundance of the bacterial taxa.

- Fungus are displaced by gut microbiota during the first 6 month of life. Primarily Candida and some Rhodotorula remain at 6 months of age.

Laforest-Lapointe *et al.* Front Immunol 2017

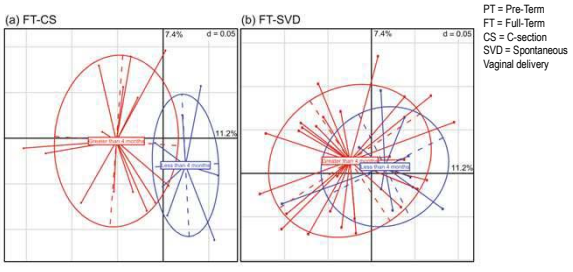
Early-life factors affecting infant gut microbiota



- Vaginal birth ensures installation of a stable gut-microbiota. C-section leads to an expansion of Firmicutes and a lack of Actinobacteria and Bacteroidetes. The profile converges to normal during the first 1-2 months of life.

Hill *et al.* Microbiome 2017

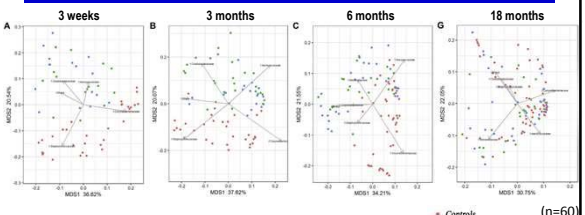
Early-life factors affecting infant gut microbiota



- Breast-feeding has bigger impact on gut microbiota composition at 24 weeks of age in C-section born infants compared to vaginal birth. Possibly, breastfeeding aids in the restoration of a "normal" gut microbiota (comparable to Vaginal Birth infants).

Hill *et al.* Microbiome 2017

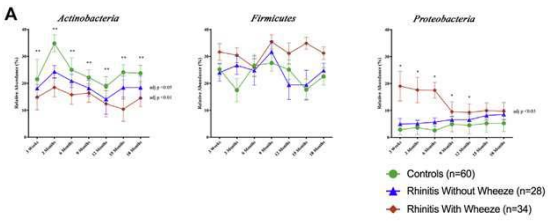
Bacterial dysbiosis and Rhinitis with or without wheeze



- Prospective birth cohort recruited in Singapore.
- **Nasal** microbiota: Multidimensional scaling using PCoA based on Bray-Curtis dissimilarity project discriminate controls and allergic individuals for early-life samples.
- Discrimination disappears with age (disruption occurs gradually from 6 to 18 months of age).

Ta *et al* JACI 2018

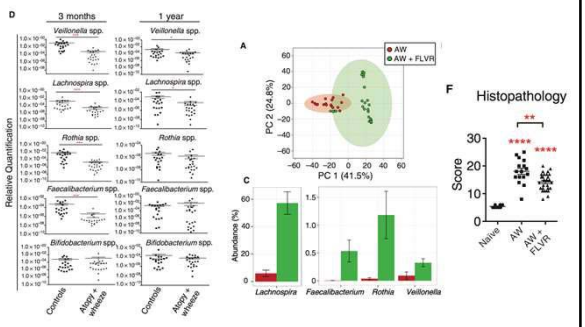
Bacterial dysbiosis and Rhinitis with or without wheeze



- Despite that discrimination based on overall nasal microbiota composition disappears with age (>6 months of age) individual abundance differences of Actinobacteria (e.g. Bifidobacterium) continue to discriminate even at late time points.
- In the intestine Actinobacteria are abundant at early time points and retract at later time points (less than 5% of healthy adult gut microbiota). Nasal content of these bacteria is less well characterized.

Ta *et al* JACI 2018

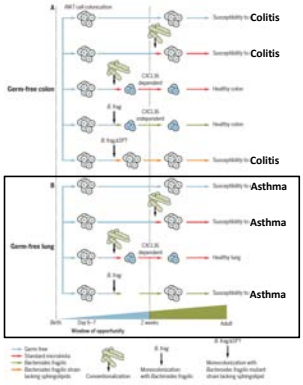
Early-life factors affecting infant gut microbiota



- **Mouse model of airway inflammation:** Gut microbiota from atopic-wheeze (AW) children drives airway inflammation. Adding 4 microbes with reduced abundance in AW children at 3 months partly rescues mice from developing airway inflammation.

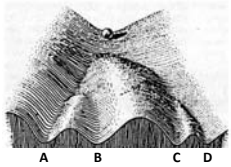
Arrieta *et al.* STM 2015

Primo-colonization - window of opportunity

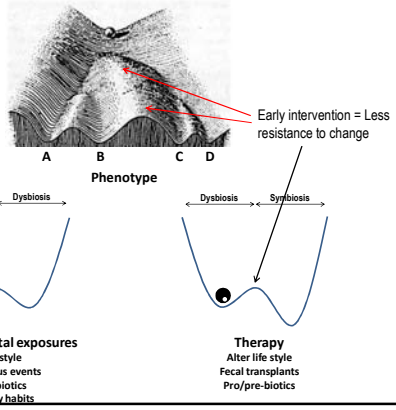


Gensollen *et al* Science 2016

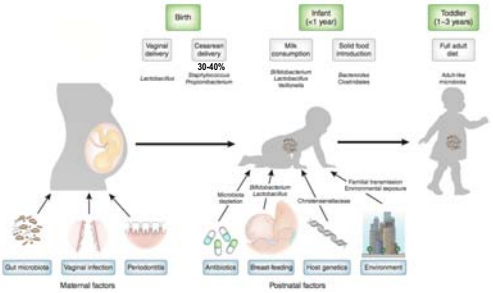
Waddington's landscape & intervention



Waddington's landscape & intervention



Early-life factors affect infant gut microbiota temporarily



- But at 1-3 years of age the child acquire an adult like gut microbiota, which is identical for all "healthy" children, independent of birth route and breastfeeding.
- If global gut microbiota is unaltered, but disease risk elevated – what can be the cause.
  - Minor persistent alterations in gut microbiota?
  - Long-term immunological memory to gut microbiota? Etc.

Tamburini *et al.* Nat Med 2016

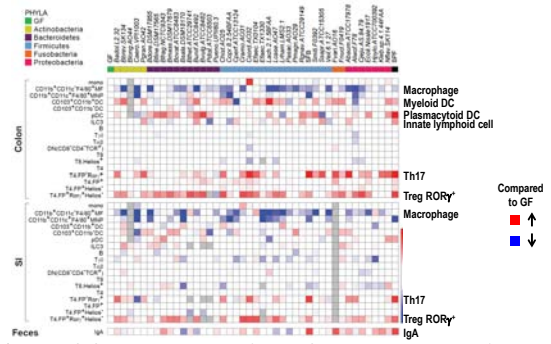
What happens to immunity?

Microbes stimulate T cell immunity

- Monocolonization of GF mice with 53 bacterial strains.
- Extensive immune phenotyping

Geva-Zatorsky *et al.* Cell 2017

Microbes stimulate T cell immunity



- APCs shifts from macrophages to DCs (more professional antigen presentation)
- Commensals increase gut residing Th17 and Treg cells.
- Phylum independent strain specific effects (e.g. free IgA in feces).

Geva-Zatorsky *et al.* Cell 2017

Temporal changes of antibody immunity

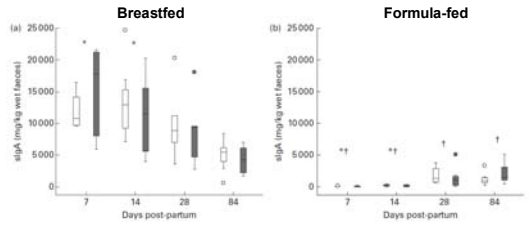
TABLE 12-12 · LEVELS OF IMMUNOGLOBULINS IN SERA OF NORMAL SUBJECTS BY AGE*

	IgG		IgM		IgA		Total Immunoglobulin	
		% of Adult		% of Adult		% of Adult		% of Adult
Age	mg/dl	Level	mg/dl	Level	mg/dl	Level	mg/dl	Level
Newborn	1031 ± 200†	89 ± 17	11 ± 5	1.1 ± 5	2 ± 3	1 ± 2	1044 ± 201	67 ± 13
1–3 mo	430 ± 119	37 ± 10	30 ± 11	30 ± 11	21 ± 13	11 ± 7	481 ± 127	31 ± 9
4–6 mo	427 ± 186	37 ± 16	43 ± 17	43 ± 17	28 ± 18	14 ± 9	498 ± 204	32 ± 13
7–12 mo	661 ± 219	58 ± 19	54 ± 23	55 ± 23	37 ± 18	19 ± 9	752 ± 242	48 ± 15
13–24 mo	762 ± 209	66 ± 18	58 ± 23	59 ± 23	50 ± 24	25 ± 12	870 ± 238	56 ± 16
25–36 mo	892 ± 183	77 ± 16	61 ± 19	62 ± 19	71 ± 37	36 ± 19	1024 ± 205	65 ± 14
3–5 yr	929 ± 228	80 ± 20	56 ± 18	57 ± 18	93 ± 27	47 ± 14	1078 ± 245	69 ± 17
6–8 yr	923 ± 256	20 ± 22	65 ± 25	66 ± 25	124 ± 45	62 ± 23	1112 ± 293	71 ± 20
9–11 yr	1124 ± 235	97 ± 20	79 ± 33	80 ± 33	131 ± 60	66 ± 30	1334 ± 254	85 ± 17
12–16 yr	946 ± 124	82 ± 11	59 ± 20	60 ± 20	148 ± 63	74 ± 32	1153 ± 169	74 ± 12
Adults	1158 ± 305	100 ± 26	99 ± 27	100 ± 27	200 ± 61	100 ± 31	1457 ± 353	100 ± 24

*The values were divided from measurements made in 296 healthy children and 30 adults. Levels were determined by the radial diffusion technique using specific rabbit antisera to human immunoglobulins.
†One standard deviation.
From Stiehm ER, Fudenberg HH. Serum levels of immune globulins in health and disease. A survey. Pediatrics 37:715, 1966.

- We are born with maternal IgG antibodies circulating our blood stream.
- IgM and IgA **serum** antibodies are virtually absent at birth and slowly increases during childhood.
- Intestinal antibodies are provided through breastfeeding.

IgA- Microbiota interactions



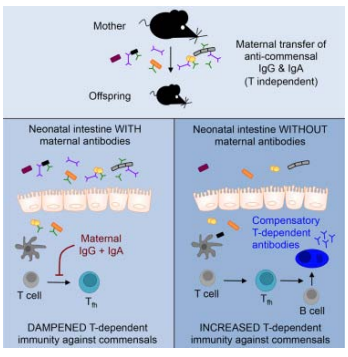
sIgA is high in breastfed infant feces and decreases over-time, likely depicting the fact that the milk becomes less rich in sIgA (colostrum (1st milk) versus breastmilk).

Formula-fed infants has initially no sIgA in their feces, but at one month post partum small amounts of autologous sIgA appears in their feces.

Women on Salmon diet (black bars) versus normal diet (white bars). Diet has no impact.

Urwin *et al.* BJ Nutrition 2014

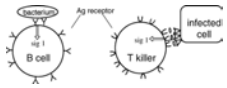
Maternal antibodies dampen offspring immunity



- T-independent (no class-switch) IgG specific for mucosal bacteria.
- Gut microbes elicit anti-commensal IgG antibodies via TLR signalling on B cells
- Maternal transmission of IgG coordinates with IgA to limit mucosal T cell responses
- Absence of maternal antibodies triggers a compensatory T-dependent immune response in the offspring.

Koch *et al.* Cell 2016

Self/non-self versus Danger model in a historical perspective

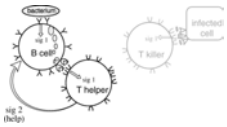


- Burnet and Lederberg propose the antigen receptor (BCR and TCR)
- Antigen stimulation (signal 1) induces immunity including class switch and somatic hyper mutation.

1950 — 1953 — 1960 — 1970 — 1980 — 1990 — 2000

Matzinger *et al.* Scan J Immunol 2003

Self/non-self versus Danger model in a historical perspective



Signal 2: e.g. CD40/CD40L and cytokines

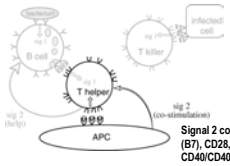
- **Problem:** BCR hypermutation may lead to autoreactive BCRs.
- **Solution:** Cohn add another cell: The T helper cell (only formally proven much later).

- B cells internalize pathogen and present antigens to interact with specific Th cells, which validate that target is non-self.
- Signal 1 alone leads to clonal deletion (both self and non-self reactivity leads to signal 1).
- Signal 1 + 2 lead to activation (non-self rescued by T cells, with TCR which does not hyper mutate).

1950 — 1960 — 1969 — 1970 — 1980 — 1990 — 2000

Matzinger *et al.* Scan J Immunol 2003

Self/non-self versus Danger model in a historical perspective



Signal 2 co-stim: CD80/86 (B7), CD28, CTLA-4, CD40/CD40L

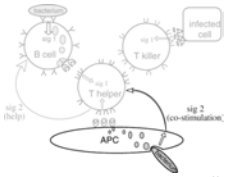
- **Problem:** We need a BCR independent manner to capture and present antigens to prime naïve T cells to become T helper cells.
- **Solution:** Lafferty and Cunningham propose that Th cells are primed and activated through APC antigen presentation and co-stimulation.

- Th cells are not constitutive active – feedback regulation upon antigen removal.
- Heavily criticized because APCs do not explain how the immune system distinguish between self and non/self (which BCR dependent antigen selection provided).

1950 — 1960 — 1970 — 1974 — 1980 — 1990 — 2000

Matzinger *et al.* Scan J Immunol 2003

Self/non-self versus Danger model in a historical perspective



- **Problem:** Contrary to B cells, APCs do not differentiate between self and non-self.
- **Solution:** Charlie Janeway proposes that APCs internalize and present non-self selectively through pattern recognition receptors (PRR), which bind elements from foreign organisms, such as bacteria.

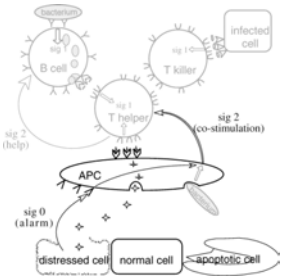
- This model inherently imply that APCs are not constitutively active, but require external stimuli through the PRR signalling pathway.
- Propose explanaition why vaccines need an adjuvant.

1950 — 1960 — 1970 — 1980 — 1989 — 1990 — 2000

Matzinger *et al.* Scan J Immunol 2003

Self/non-self versus Danger model in a historical perspective

Problem
How to explain:
- Autoimmunity
- Non-reject of tumour with tumour antigen
- DNA therapy versus DNA vaccine
- Why mothers don't reject the fetus.
- Why temporal gene-expression changes doesn't evoke immunity (e.g. breast milk).
- Why can we host tons of microbes?



- **Solution:** Polly Matzinger proposes that APCs are activated through danger signals from any distressed tissue (uric acid, heparin sulfate, extracellular ATP and DNA/RNA).

- Advocate that we are a friendly host as long as our visitors are friendly too. Don't push the button first policy.

1950 — 1960 — 1970 — 1980 — 1990 — 1996 — 2000

Matzinger *et al.* Scan J Immunol 2003

Danger model and gut microbiota symbiosis

- The danger model would suggest that we do not respond to non-harmful bacteria colonizing our gut (Mutualism and Commensalism allowed).

Symbiosis
Living together (sym: with; bio: life)

Danger

Mutualism	Commensalism	Pathobiont	Parasitism
Both benefit	One benefit; one not harmed	Both benefit if regulated	One benefit; one harmed
Immune Ignorance (no immunity)		????	Cytotoxic Immunity

Danger model and IgA responses

- The danger model would suggest that we do not respond to non-harmfull bacteria colonizing our gut (Mutualism and Commensalism allowed).

Symbiosis
Living together (sym: with; bio: life)

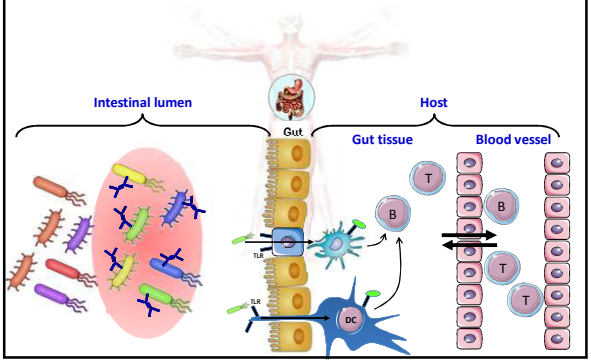
Danger — IgA

Danger — No IgA

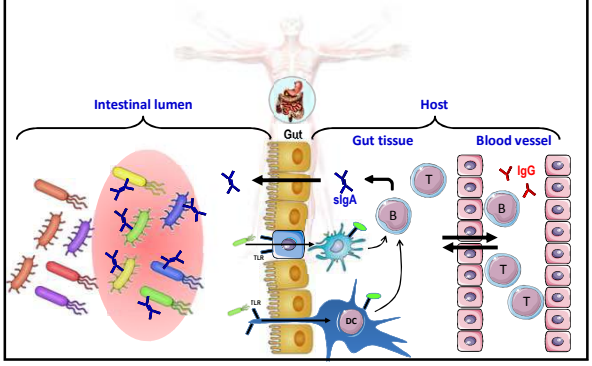
Mutualism	Commensalism	Pathobiont	Parasitism
Both benefit	One benefit; one not harmed	Both benefit if regulated	One benefit; one harmed
Immune Ignorance (no immunity)		Immune Tolerance (e.g. IgA, Treg)	Cytotoxic Immunity

- In reality microbes cannot be categorized discretely, but rather represents a continuum from Mutualism to parasitism.
- How to retain tolerance to commensals, while pathogens are attacked?
- The range of host-microbe interactions evokes ignorance to non-harmful microbes, tolerogenic immunity to beneficial microbes (harmful if not regulated) and cytotoxic immunity to harmful microbes.

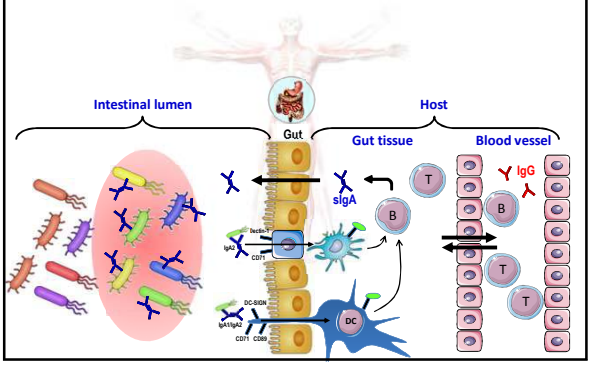
Interactions between host and gut microbiota



Interactions between host and gut microbiota

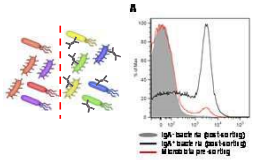


Interactions between host and gut microbiota



Gut microbiota specificity of gut Ig immunity

Gut microbiota sorting (IgA⁺ / IgA⁻)

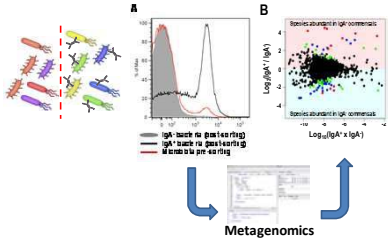


Moor ... Larsen. Nature Prot 2016

Gut microbiota specificity of gut Ig immunity

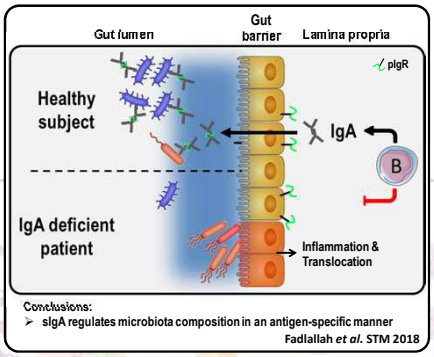
Gut microbiota sorting (IgA⁺ / IgA⁻)

Paired metagenomic analysis



Fadlallah, El-Kafsi, ... Larsen. STM 2018

Interactions between host and gut microbiota



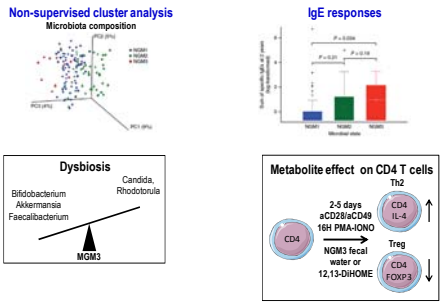
Conclusions:

- sIgA regulates microbiota composition in an antigen-specific manner

Fadlallah *et al.* STM 2018

Examples of gut microbiota associations with allergy

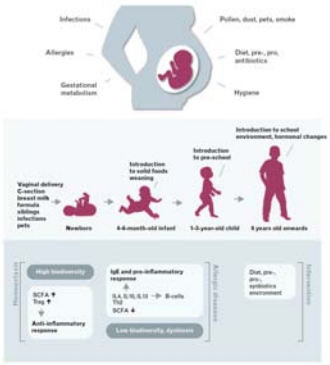
Gut Microbiota composition is associated with allergic disease



- Neonatal gut microbiota (NGM) clusters differentiate low and high risk allergic disease (loss of bacteria and gain of fungus increase risk of allergy).
- Dysbiosis alters the metabolic profile of fecal water (reduction of Tregs – increase in Th2 cells).

Fujimura *et al.* Nat Med 2016

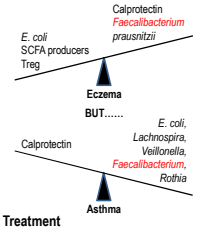
Gut microbiota and eczema



Predictive markers:

High fecal calprotectin (inflammation) and low abundance of fecal E. coli at 2 months predicted asthma and eczema by the age of 6 years.

Ruminococcaceae reduced at 1 week of age in infants that developed IgE-associated eczema.

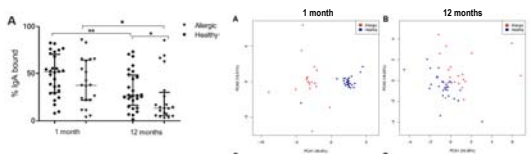


Treatment

Pre- and probiotics have effect in mice, but so-far not convincingly in humans.

Sjodin *et al* Curr Opin Allergy & Clin Immunol 2016

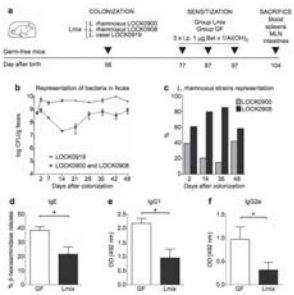
Gut Immuno-Microbiome - allergic disease



- Allergy is associated with reduced sIgA-opsonization levels of gut microbiota in 12 months-old children (tendency already at 1 month).
- Fecal sIgA levels are unaltered.
- Gut bacterial load is reduced at 12 months of age in allergic children
- A unique immuno-microbiome signature precedes allergy already at 1 month of age.
- sIgA-opsonization and immuno-microbiome signature are similar when stratified for birth route and breastfeeding (Of note, breastfeeding is assessed at 12 months – practically all Swedish children are breastfed in the first months of life)

Dzidic *et al.* JACI 2016

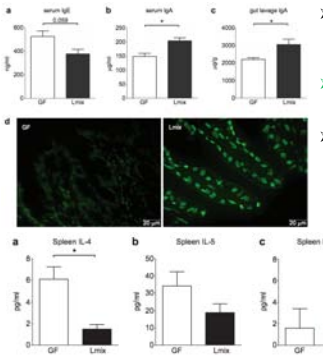
GF vs Lactobacillus colonization - Birch pollen sensitization



- Lactobacillus strains enhance gut barrier integrity (ZO-1; not shown) and create tolerance to birch pollen sensitization (reduced antibody responses to Bet v1)

Kozakova *et al.* Cell Mol Biol 2016

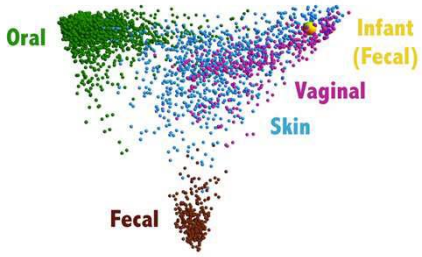
GF vs Lactobacillus colonization - Birch pollen sensitization



- Total antibody titers are shifted from IgE to IgA in lactobacillus colonized mice compared to GF.
- IgA⁺ B cells (green) are absent in the small intestine of GF mice.
- Cytokine levels in the spleen shifts from Th2 to Th1 type cytokines.

Kozakova *et al.* Cell Mol Biol 2016

Gut microbiota maturation during first 2 years of life



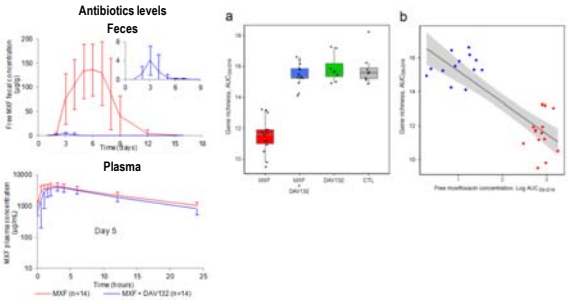
Dr. Rob Knight

Avoid intestinal effect of antibiotics

WARNING – ADVERTISEMENT!

De Gunzburg *et al* JCI 2017

Avoid intestinal effect of antibiotics



- Antibiotics administered orally for non-intestinal infections (dental, sinus, pulmonary etc.) are taken up primarily in the stomach. Remains pas through our lower GI tract.
- Activated coal (DAV132) absorbs/inactivates 99% of antibiotics (e.g. Moxifloxacin, MXF).
- DAV132 retains normal antibiotic plasma levels, but reduces fecal levels and protects gut microbiota from antibiotic induced dysbiosis.

De Gunzburg *et al* JCI 2017

Take home message

- Gut microbiota influence host immunity (may skew immunity towards Th2 and IgE – to be confirmed?)
- Gut microbiota is regulated by host immunity (innate and adaptive (e.g. IgA))
- **Altered lifestyle** influence our gut microbiota composition and is temporally (but maybe not causally) associated with a rapid increase in chronic inflammatory diseases, including allergy (since 1950 forward).
- **Hygiene theory**: Reduced exposure to microbes result in a skewed host immunity, which has not been sufficiently schooled to regulate inflammatory responses.
- **Save our microbiota:** Vaginal microbiota transplantation (C-section birth), reduce antibiotics use (or use of new treatments, such as DAV132 co-therapy).
- **Save our immunity:** Probiotics (do not colonize), helminths (worms), immune therapy (allergy), promote breast feeding.