Centre d'Immunologie et des Maladies Infectieuses

LU3SV649
Microbiota ecology and host immunity

Martin LARSEN

INSERM U1135, CHU Pitié-Salpetrière, Paris, France

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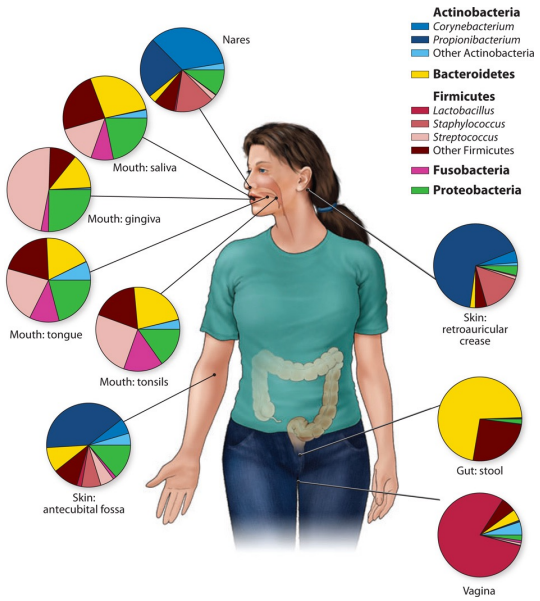
Human body sites harbor distinct microbiota



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

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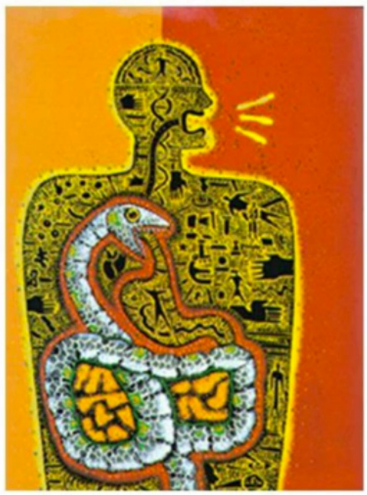
The human Gut and its inhabitants in numbers



- 30 tons of food and 50.000 L during a lifetime
- Huge mucosal surface:30-40 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).
- $5x10^{13}$ bacteria: 3x number of cells in the entire body, i.e. 1-2 kg.
- 100 times more bacterial genes than human genes.

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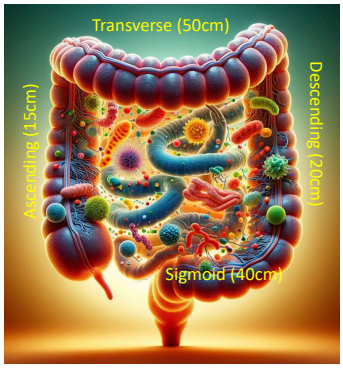
The digestive system - A trip through the GI tract

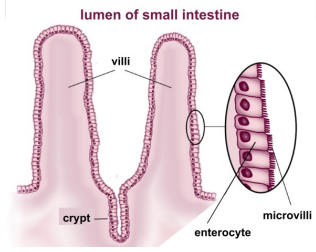
Small intestine
(duodenum -> jejunum -> ileum)

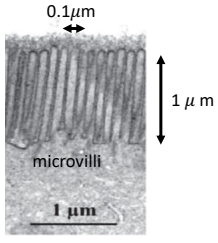
- 3-4m long
- 2.5cm diameter
- Has folds (2x) and villi (8x)
- Has microvilli (8x)
- Surface: 30-40m^2
- pH < 7
- Some oxygen
- Host low diversity microbiota.
- Retrieves nutrients

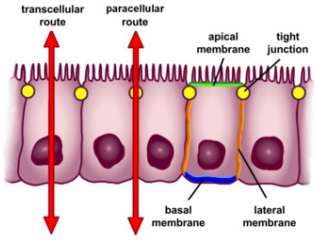


Large intestine
(cecum -> colon -> rectum -> anus)

- 1.5m long
- 4.8cm diameter
- Has no folds nor villi
- Has microvilli (10x surface)
- Surface: 2m^2
- pH = 7
- Anaerobic
- Host diverse microbiota.
- Retrieves water and salts.
- Degrades complex nutrients







Helander et al. Sc J Gastro 2014

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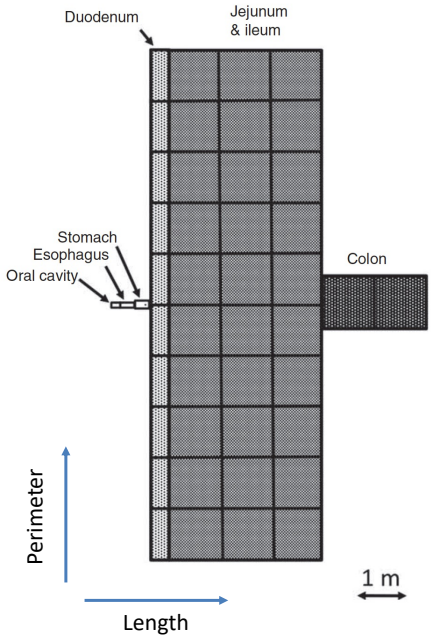
The digestive system – Anatomy revisited

Small intestine:

Textbook
- **Length: 22m**
- **Diameter: 2.5cm**
- **Surface: 150-200m² (Tennis court)**

Revisited:
- **Length: 3-4m**
- **Diameter: 2.5cm**
- **Surface 30-40m² (half a badminton court)**

Big problems with the measures, because you can mechanically stretch the intestine during e.g. an autopsy.



Helander et al. Sc J Gastro 2014

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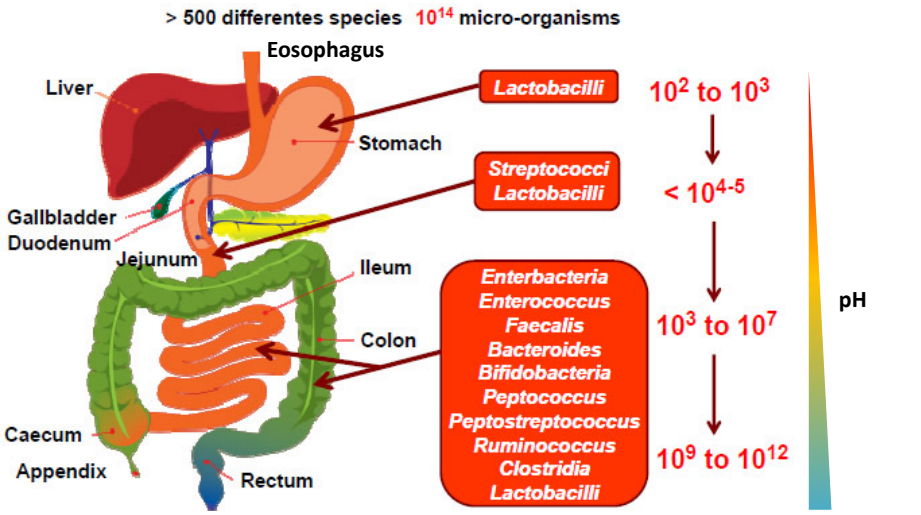
Spatial distribution of gut microbiota



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

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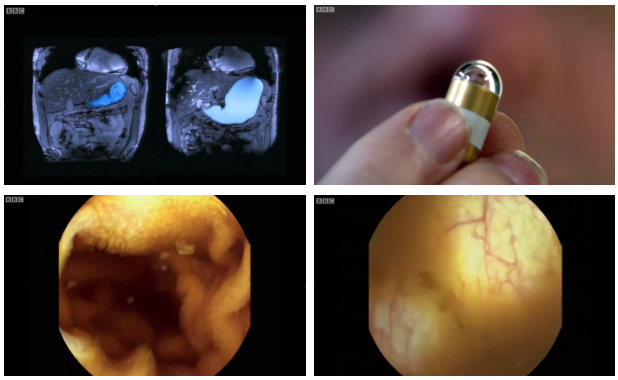
The digestive system - A trip through the GI tract



- 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!**

Watch video: http://www.immulab.fr/cms/index.php/teaching/immunology-teaching

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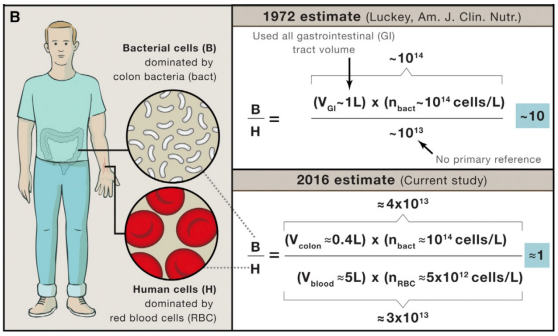
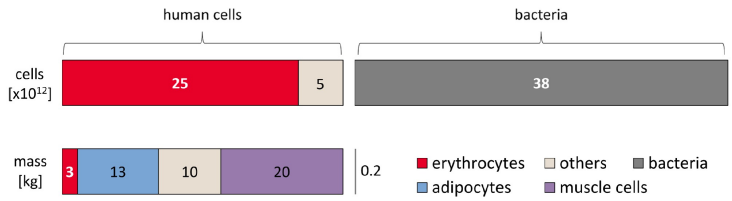
We are outnumbered and outsmarted



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

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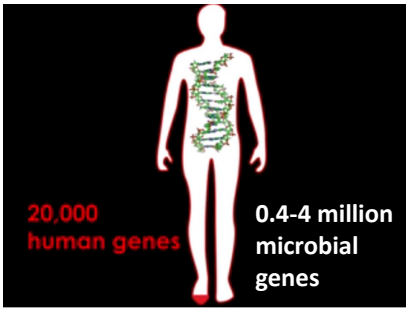
We are outnumbered and outsmarted

Outsmarted
(20-200 fold more genes)



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

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How to study the gut microbiota

- Culture dependent (classic microbiology – aerobiosis/anaerobiosis, medium)
  - Microscopy
  - Metabolism of substrates
  - Co-culture with host cells (epithelium, immune cells etc.)
  - Genetic modifications
  - Culturomics – strain isolation (e.g. FACS)
  - Antibody titers to microbes (ELISA, flow cytometry)

- Culture independent
  - Mass spectrometry
  - Flow cytometry (FISH, immuno-microbiota)
  - Metabolomics
  - Metagenomics (microbiome and immuno-microbiome)
  - Metatranscriptomics

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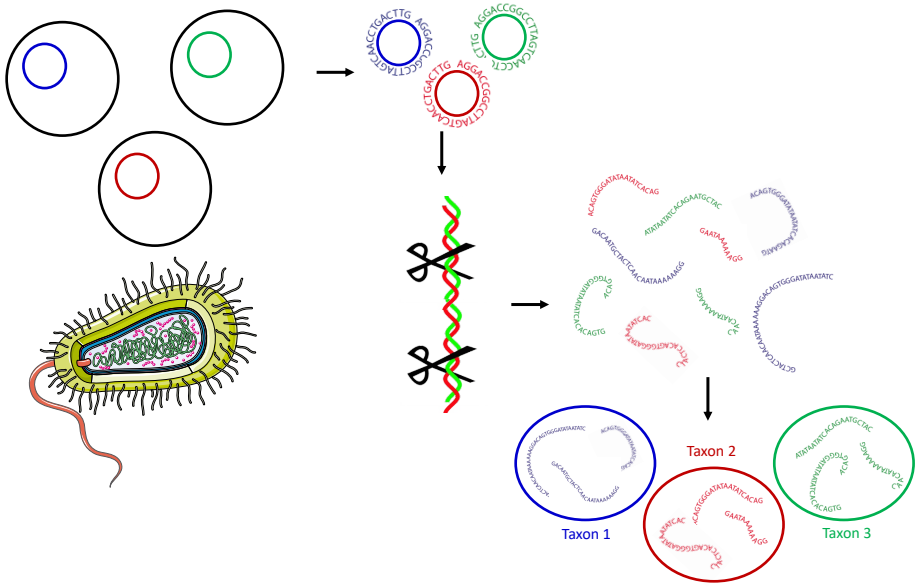
Next Generation Sequencing - like reading a threaded book



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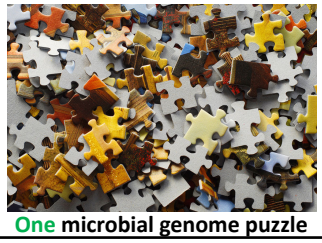
Microbial whole genome sequencing

? assembly

- One microbe is fully sequenced and assembled.



One microbial genome puzzle

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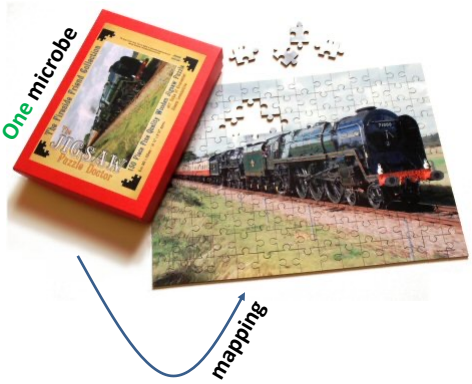
Microbial whole genome sequencing



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

One microbial genome puzzle

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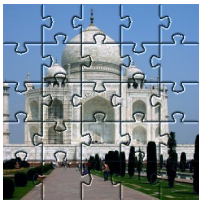
Metagenomic sequencing



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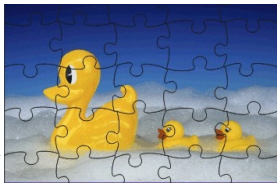

- A mixture of many microbes

Mixture of microbial genomes

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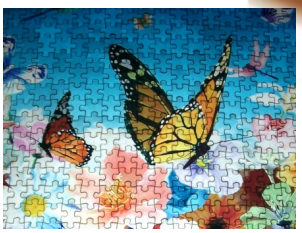
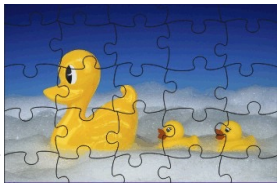
Metagenomic sequencing



mapping



- 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

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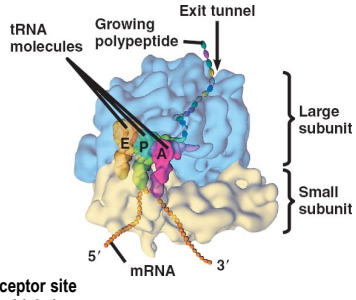
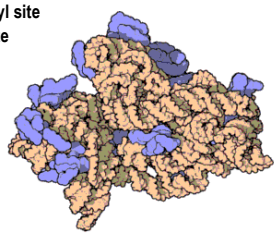
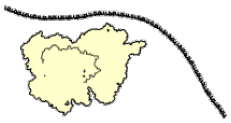
16S rRNA sequencing



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

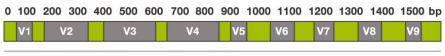


- 16S rRNA in orange
- Associated protein subunits in blue

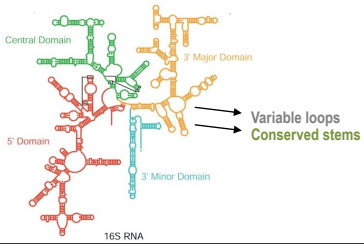


Protein synthesis in the
1. Cytosol
2. Endoplasmic reticulum
   ER-docking via signal-recognition particle

16S rRNA primary and secondary structures



CONSERVED REGIONS: unspecific applications
VARIABLE REGIONS: group or species-specific applications



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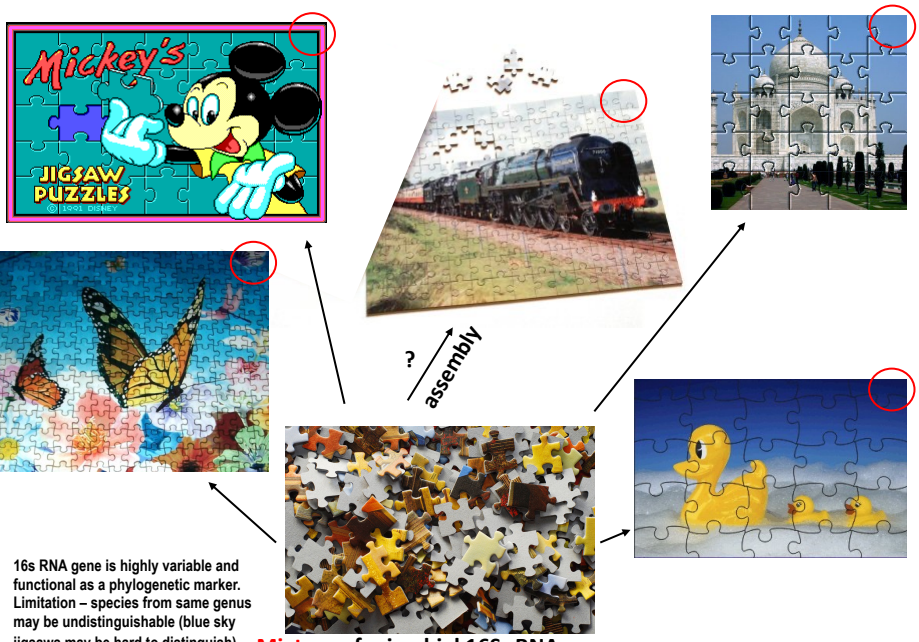
16S rRNA sequencing



- 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

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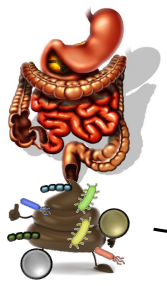
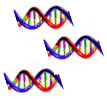
Big data in Microbiology

Intestinal tract

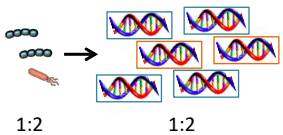


Genomic DNA



Next Generation Sequencing

Identification:
1. Ref. genome
2. Co-abundance clustering



1:2 1:2

MetaGenomic Species (MGS)

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

Nielsen *et al.* Nat Biotech 2014

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Alpha-diversity

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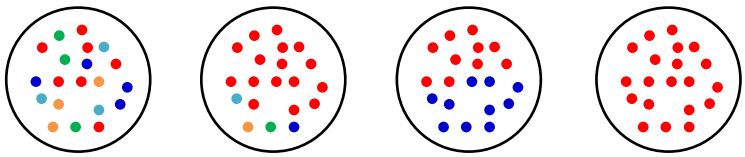
Alpha-diversity

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	5	5	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_1=1 H=0 [E_H=0]

- Scores of alpha-diversity: Richness, Shannon, Simpson, Chao1 and Chao2
- Chao1 and Chao2 often used in microbiota research. Used when certain species are rare (based on expected rather than observed number of species in a sample).

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Beta-diversity

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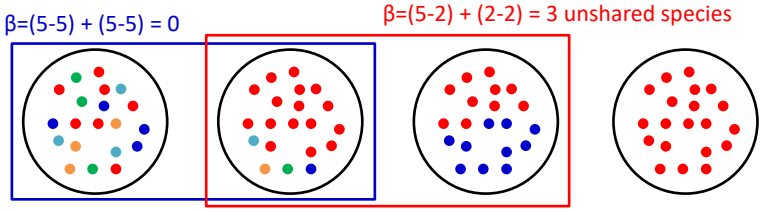
Beta-diversity

- Total species diversity (γ) is determined by the mean species diversity of a habitat (α) plus the differentiation among habitats (β). **Robert Whittaker, 1960**
- Pair-wise beta-diversity is measured as similarity or dissimilarity between two samples.
- Beta-diversity is also referred to as species turn-over.
- Absolute species turn-over: $\beta=(R_1-c_{12}) + (R_2-c_{12})$, R=Richness, c=common species



Species richness	5	5	2	1

- Absolute species turn-over is problematic when working with rare species, whose occurrence is associated with random sampling efficiency. Abundance based measures are therefore more appropriate for microbiota work.
- Scores of beta-diversity:
- Occurrence based: Absolute species turn-over, Whittaker species turn-over (special case of Sørensen similarity index) and Proportional species turn-over (Jaccard similarity index).
- Abundance based: Bray–Curtis dissimilarity index, Morisita-Horn overlap index.

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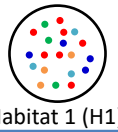
Beta-diversity

Occurrence based Beta-diversity



	Habitat 1 (H1)	H2	H3	H4
H1	0			
H2	0	0		
H3	$\beta_{3,1} = 3$	$\beta_{3,2} = 3$	0	
H4	4	4	$\beta_{4,3} = 1$	0

- One could argue that H1 and H3 are more distant ($\beta_{3,1}$) than H2 and H3 ($\beta_{3,2}$), which should be closer to $\beta_{4,3}$. This would be better represented by an abundance based beta diversity.

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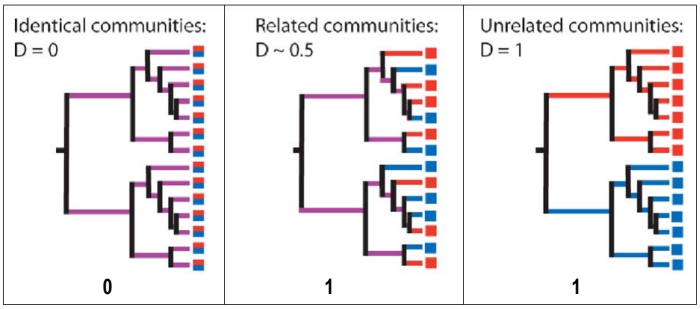
Beta-diversity

- Classic beta-diversity measures consider all genetically different species equally different (*E. coli* and *K. pneumoniae* (both Proteobacteria) are as different as *E. coli* and *B. longum (Actinobacteria)*).
- Species from same phylum, class, order, family or genus are more phylogenetically similar than unrelated bacteria.
- Solution: UniFrac

Unique Fraction (UniFrac) metric

- Qualitative phylogenetic β diversity.
- Distance = fraction of the total branch length that is unique to any particular environment.

Occurance based: Normalized species turn-over:



Lozupone and Knight, 2005, Appl Environ Microbiol 71:8228

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Early-life microbiota colonization

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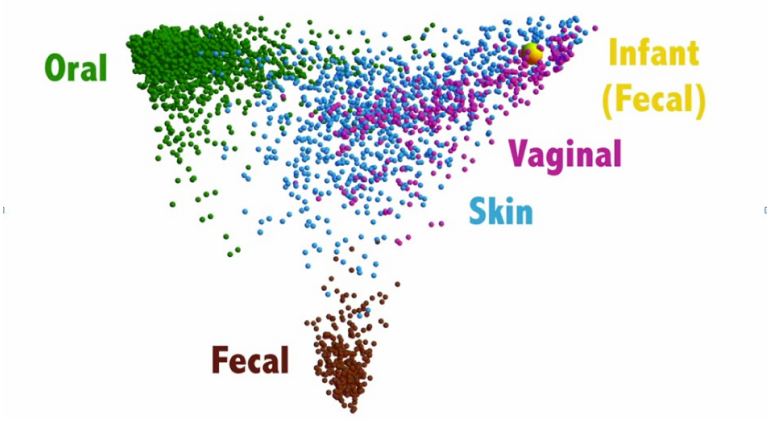
Gut microbiota maturation during first 2 years of life



How to avoid deleterious effect of antibiotics on gut microbiota, while preserving systemic anti-microbial activity?

Watch video: http://www.immulab.fr/cms/index.php/teaching/immunology-teaching

Dr. Rob Knight

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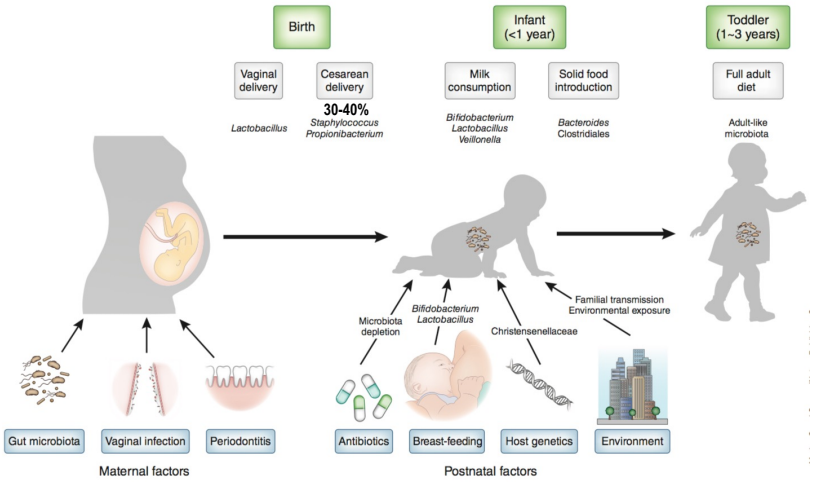
Early-life factors affecting infant gut microbiota



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

Tamburini *et al.* Nat Med 2016

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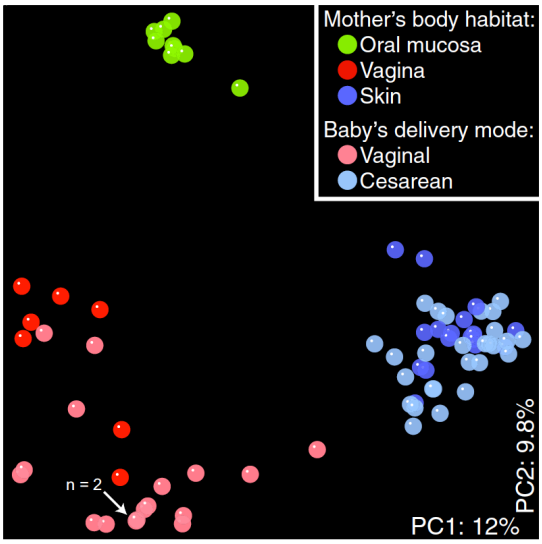
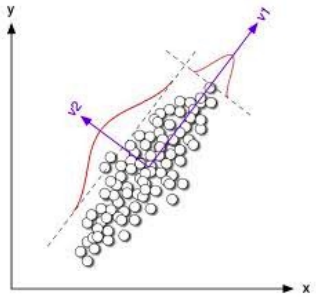
Delivery mode shape early life gut microbiota colonization



Principal component analysis
Define axis representing maximal data variance!!



Dominguez-Bello *et al* PNAS 2010

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Fecal Microbial Transfer (FMT) rescue microbiota post c-section.

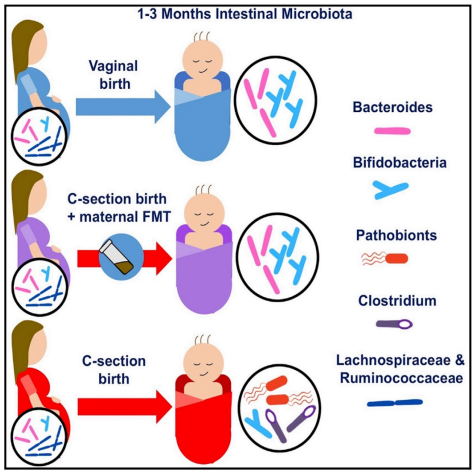
Cell

Article

Maternal Fecal Microbiota Transplantation in Cesarean-Born Infants Rapidly Restores Normal Gut Microbial Development: A Proof-of-Concept Study

- Fecal microbiota development of newborns is dependent on the mode of delivery
- The development in cesarean section-born infants deviates from that of vaginally born infants
- This deviation can be prevented by fecal microbiota transplantation from the mother (T3 feces; collected 3 weeks prior to delivery)
- Transplanted cesarean section-born infants show normal fecal microbiota development at 1-3 months of age.

7 infants received FMT
(1 time 3.5mg = 10^6-10^7 bacteria)



Korpela *et al.* Cell 2020

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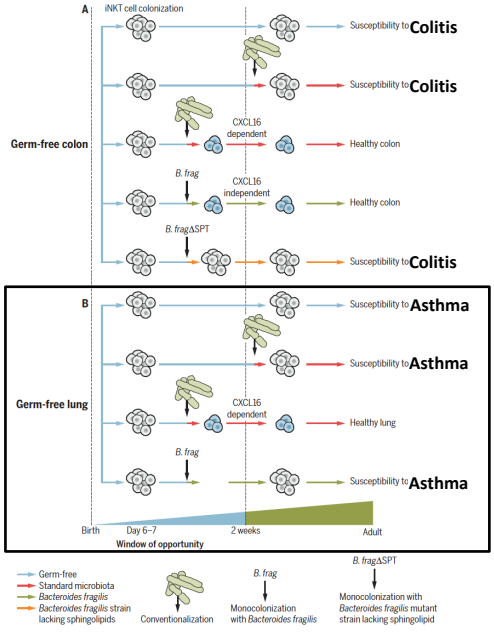
Primo-colonization - window of opportunity



Gensollen *et al* Science 2016

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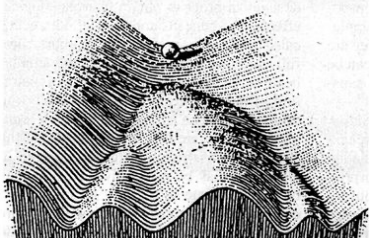
Waddington's landscape & intervention



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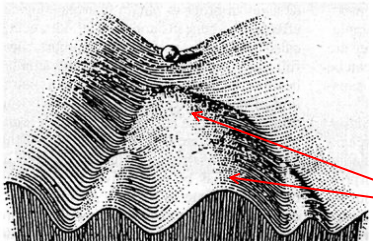
Waddington's landscape & intervention

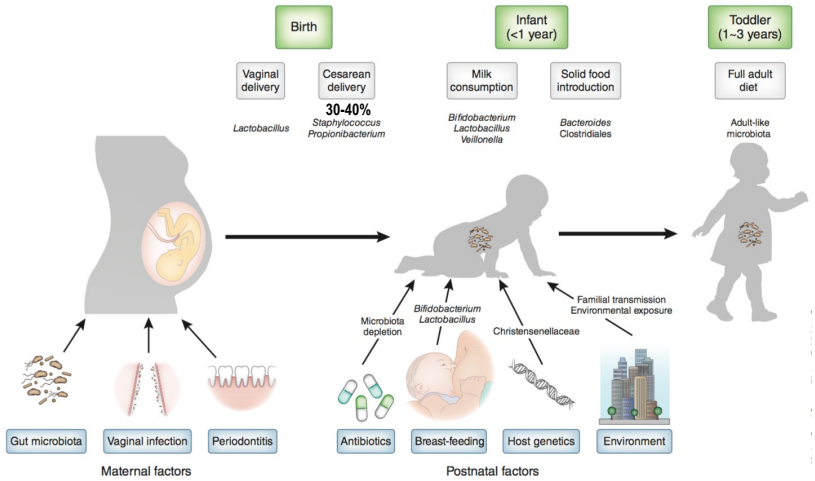


Environmental exposures
Life style
Infectious events
Antibiotics
Dietary habits

Therapy
Alter life style
Fecal transplants
Pro/pre-biotics

64

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

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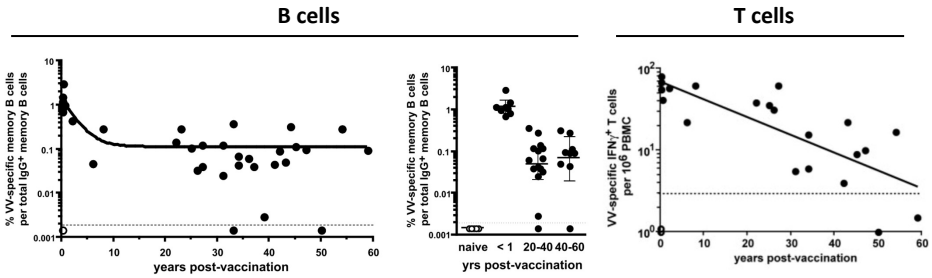
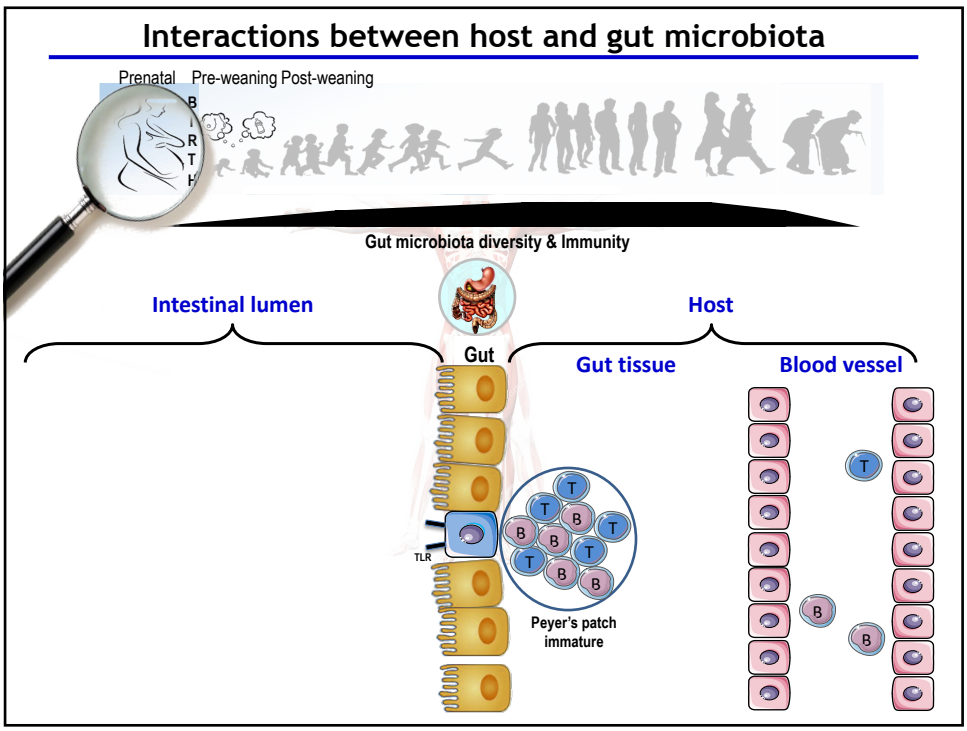
Temporal changes of antibody immunity



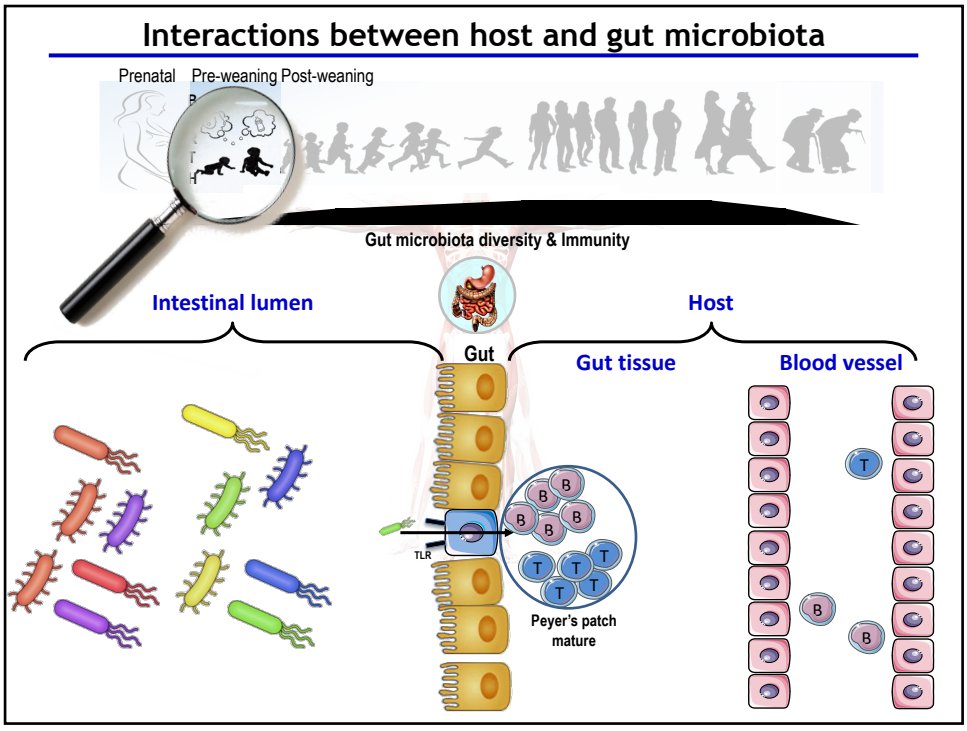
- VV = Vaccinia Virus (Smallpox = "Variole")
- B cell retraction phase is followed by long-term (+50 years) stable maintenance of B cell memory.
- T cells continue to retract, but remain detectable for more than 50 years.

Crotty *et al.* JI 2003

74


Interactions between host and gut microbiota
Prenatal
Pre-weaning
Post-weaning
BIRTH
Gut microbiota diversity & Immunity
Intestinal lumen
Host
Gut
Gut tissue
Blood vessel
TLR
Peyer's patch
immature

88


Interactions between host and gut microbiota
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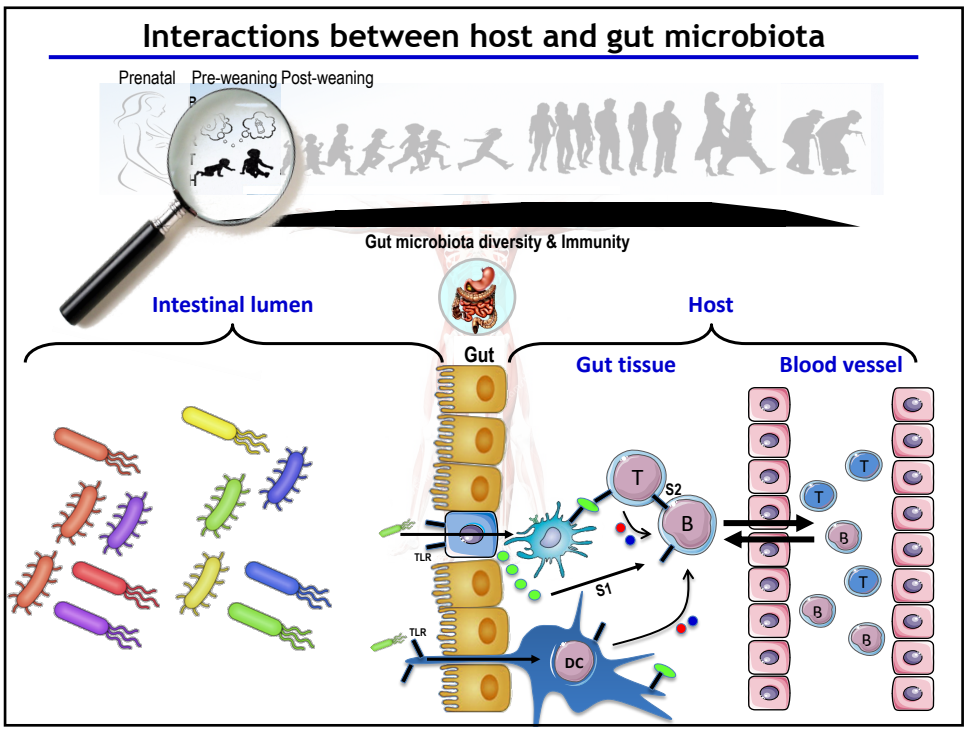
Post-weaning
Gut microbiota diversity & Immunity
Intestinal lumen
Host
Gut
Gut tissue
Blood vessel
TLR
Peyer's patch
mature

89


Interactions between host and gut microbiota
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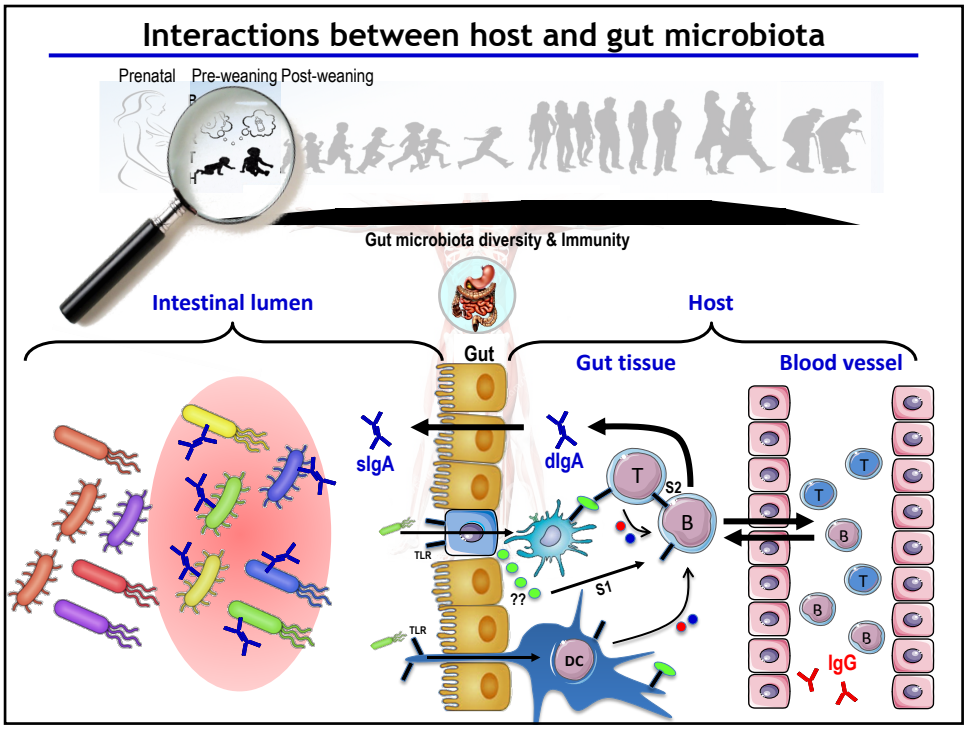
Post-weaning
Gut microbiota diversity & Immunity
Intestinal lumen
Host
Gut
Gut tissue
Blood vessel
TLR
TLR
T
S2
B
S1
DC

90


Interactions between host and gut microbiota
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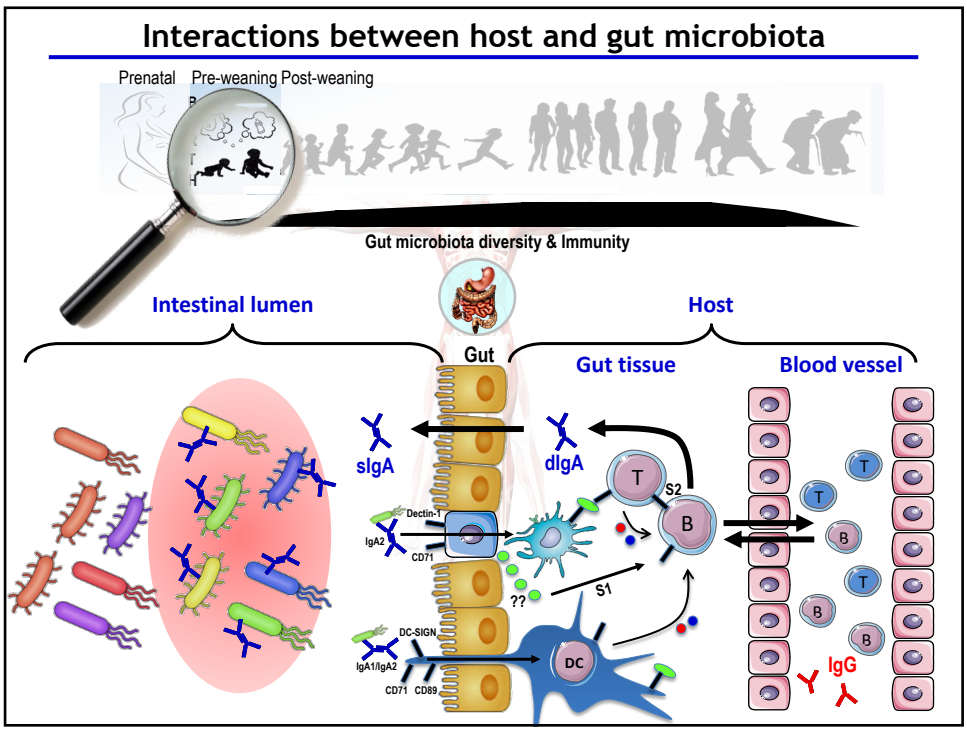
Pre-weaning
Post-weaning
Gut microbiota diversity & Immunity
Intestinal lumen
Host
Gut
Gut tissue
Blood vessel
sIgA
dIgA
TLR
TLR
??
T
S2
B
S1
DC
IgG

91


Interactions between host and gut microbiota
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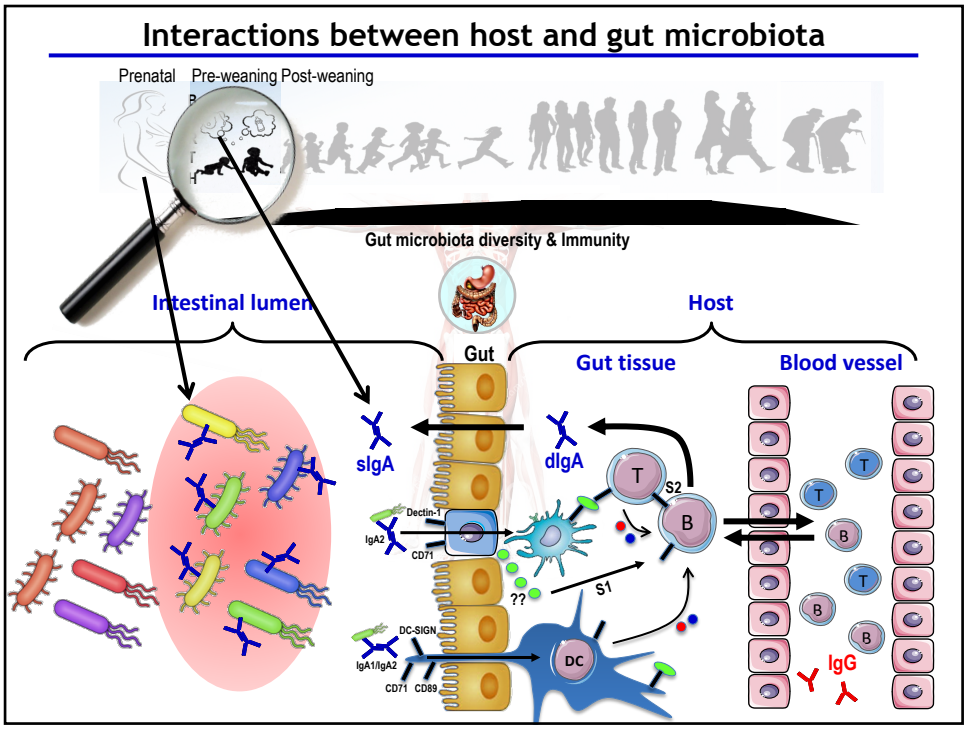
Intestinal lumen
Host
Gut
Gut tissue
Blood vessel
sIgA
dIgA
T
S2
B
Dectin-1
IgA2
CD71
??
S1
DC-SIGN
IgA1/IgA2
CD71
CD89
DC
IgG

92


Interactions between host and gut microbiota
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CD71
CD89
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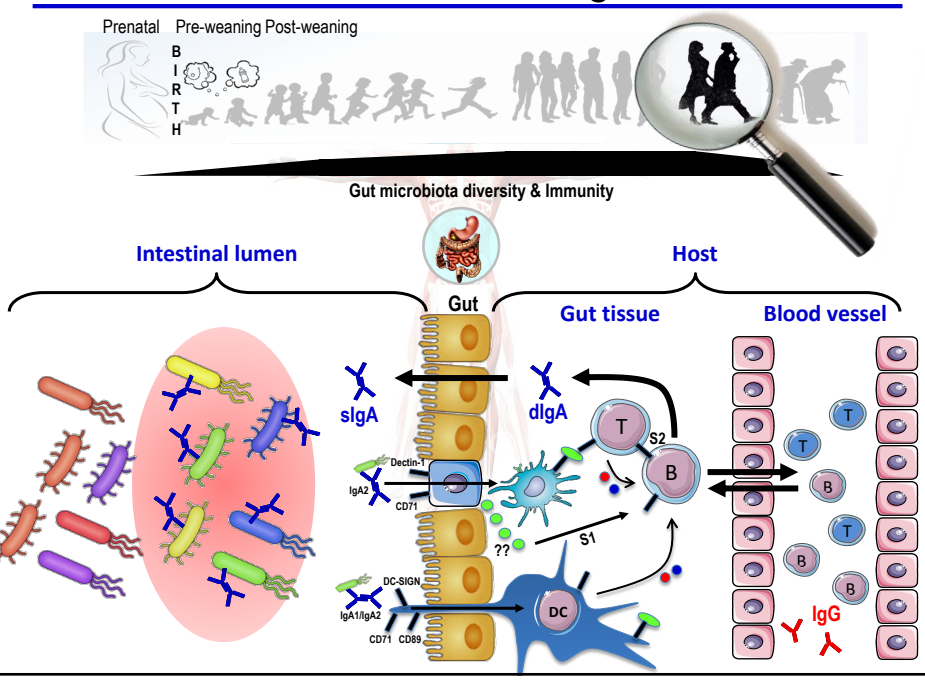
IgG

93

Interactions between host and gut microbiota



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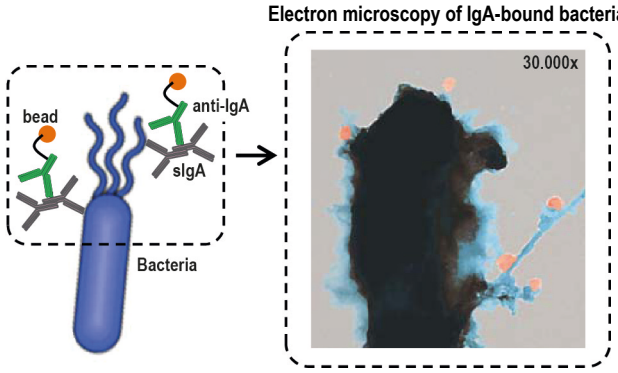
Gut microbiota specificity of gut Ig immunity

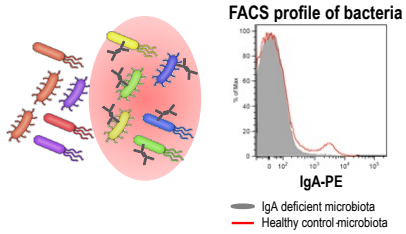
Gut microbiota immuno-monitoring



97

Gut microbiota specificity of gut Ig immunity

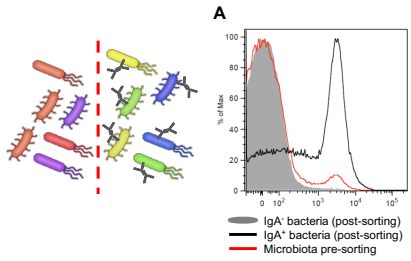
Gut microbiota immuno-monitoring



98

Gut microbiota specificity of gut Ig immunity

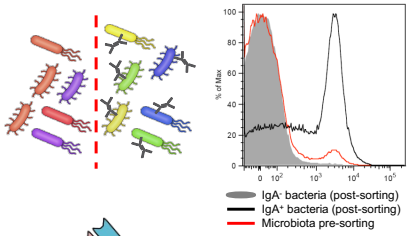
Gut microbiota sorting (IgA^+ / IgA^-)

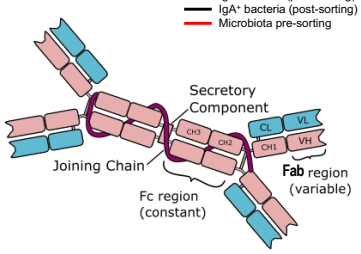


99

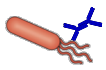
Gut microbiota specificity of gut Ig immunity

Gut microbiota sorting (IgA$^+$ / IgA$^-$)





Frequently IgA-bound microbes

Streptococcus *Staphylococcus*		Fc
Faecalibacterium *Akkermansia* *Bifidobacterium*		?
Coprococcus comes *Ruminococcus gnavus*		Fab Superantigen
Escherichia *Salmonella*		Fab

Bunker *et al.* STM 2019, Fadlallah *et al.*. STM 2018

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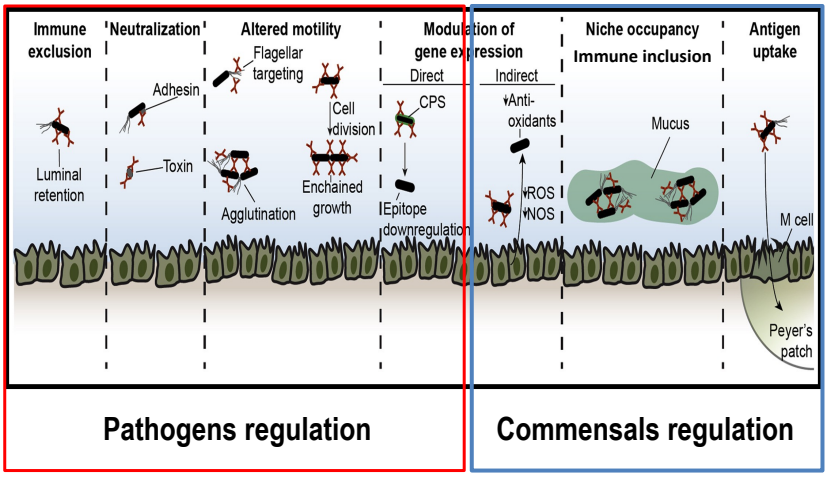
IgA mediated microbiota regulation



Bunker *et al.* Immunity 2018

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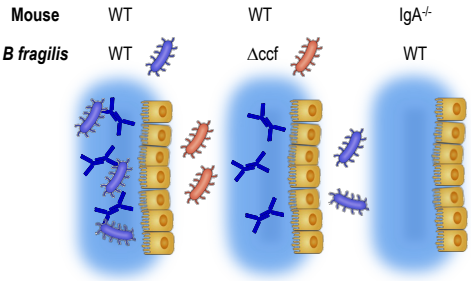
IgA mediated microbiota regulation

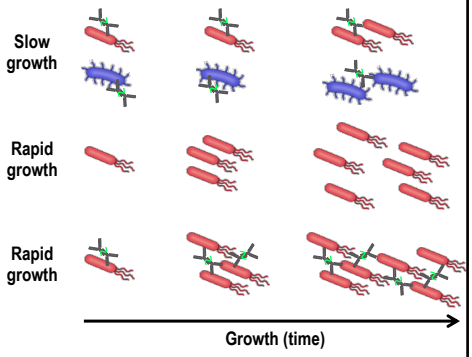
Immune-inclusion:



- *B fragilis* gut colonization regulated by sIgA : Absence of
  1. Antibody (sIgA) and/or
  2. Antigen (Capsular Polysaccharides) prohibit colonization.

Donaldson *et al.* Science 2018

Immune-exclusion:



- Phylogenetically related pathogenic and commensal microbial strains can be targeted by the same sIgA, but immune-exclusion would be specific to the pathogenic rapidly growing microbe.

Moor *et al.* Nature 2017

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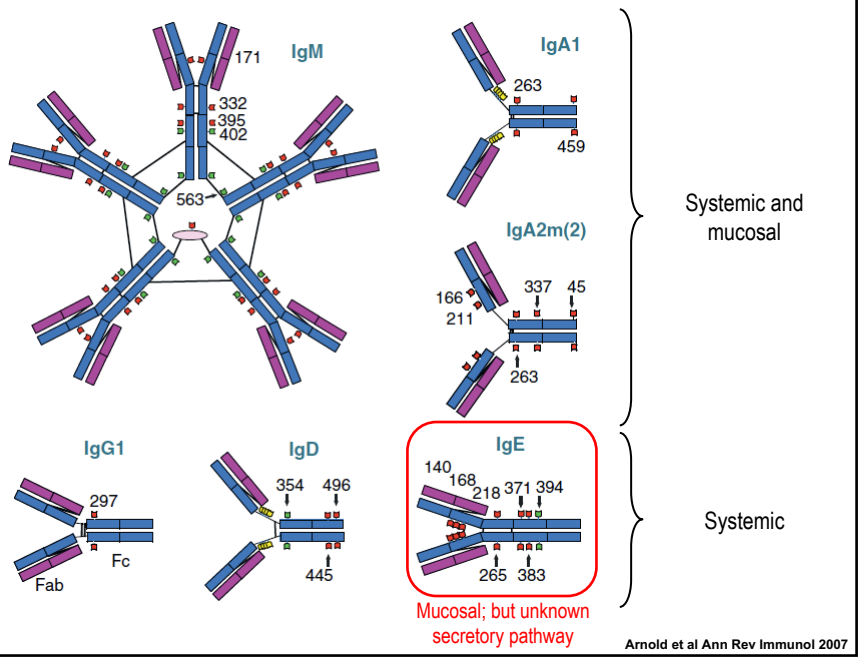
Antibody characteristics



Arnold et al Ann Rev Immunol 2007

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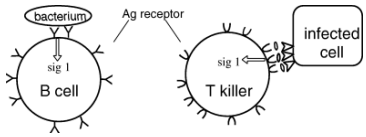
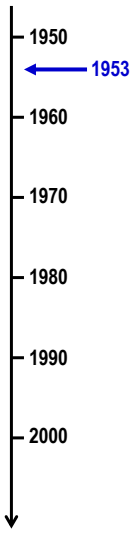
Self/non-self versus Danger model in a historical perspective





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

Matzinger *et al.* Scan J Immunol 2003

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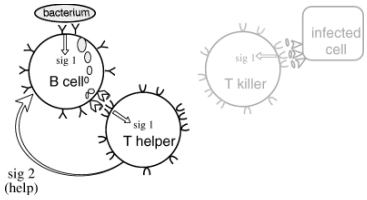
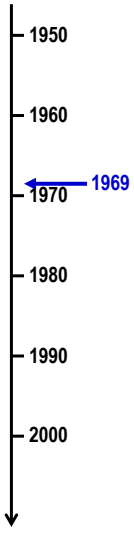
Self/non-self versus Danger model in a historical perspective



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 (Mosmann *et al.* 1986).
- 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).

Matzinger *et al.* Scan J Immunol 2003

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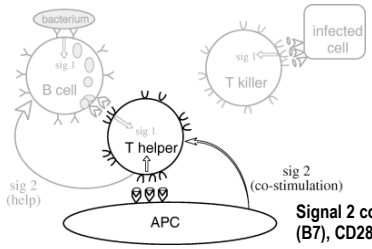
Self/non-self versus Danger model in a historical perspective



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

1950
1960
1970
1974
1980
1990
2000

- **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).

Matzinger *et al.* Scan J Immunol 2003

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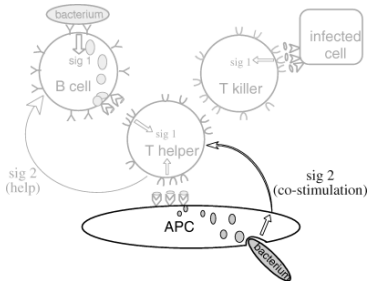
Self/non-self versus Danger model in a historical perspective



1950
1960
1970
1980
1989
1990
2000

- **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.
- Proposes explanation for why vaccines need an adjuvant.

Matzinger *et al.* Scan J Immunol 2003

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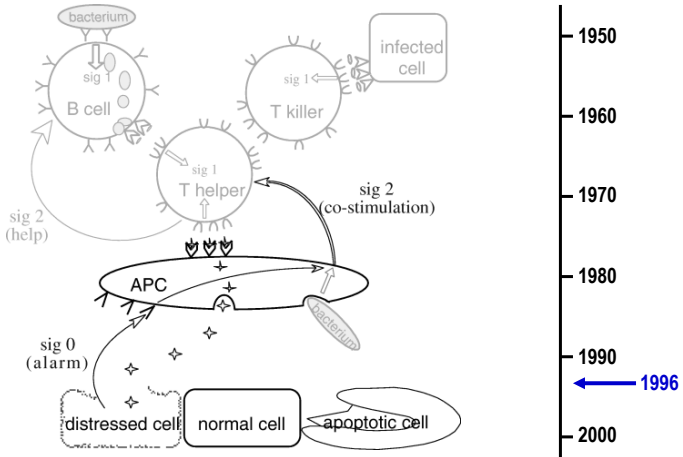
Self/non-self versus Danger model in a historical perspective

Problem

How to explain:

- Autoimmunity
- Non-reject of tumour with tumour antigen
- Why mothers don't reject the fetus.
- Why temporal gene-expression changes doesn't evoke immunity (e.g. breast milk).
- Why can we host billions 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.

Matzinger *et al.* Scan J Immunol 2003

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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 but barrier)		**????**	**Cytotoxic Immunity**

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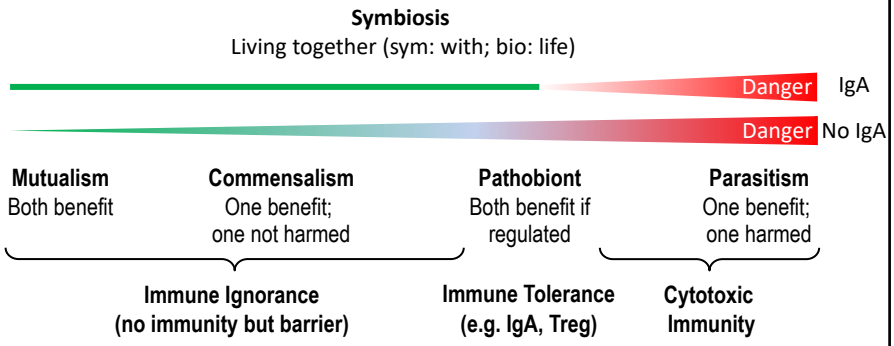
Danger model and IgA responses

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



- 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.

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Examples of gut microbiota associations with pathology

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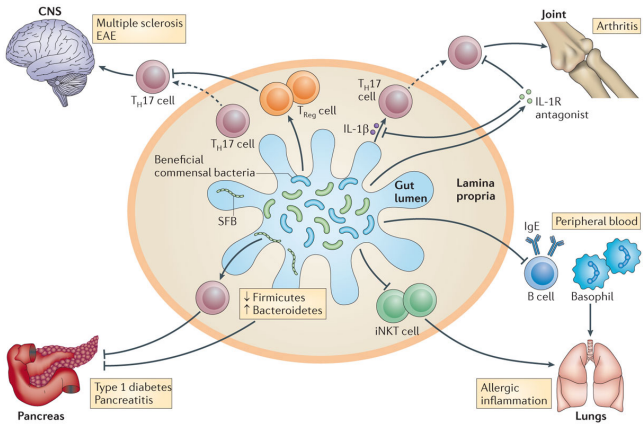
Gut microbiota and immune pathology

Autoimmune and allergic diseases 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

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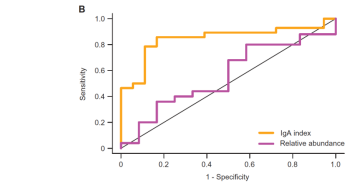
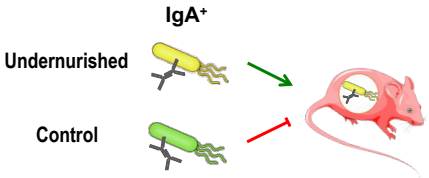
IgA targets bacteria involved in human pathology

➢ **Undernourished Malawian children produce diet-dependent enteropathy.**

➢ **IgA coated gut microbiota from children is predictive of nutritional status and enteropathy.**

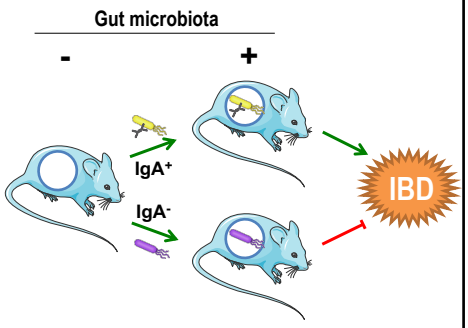




Kau *et al.* STM 2015

➢ **Mouse model of microbiota-driven colitis.**

➢ **Human IgA coated gut microbiota from IBD patients confer susceptibility to colitis in germ-free mice.**



Palm *et al.* Cell 2014

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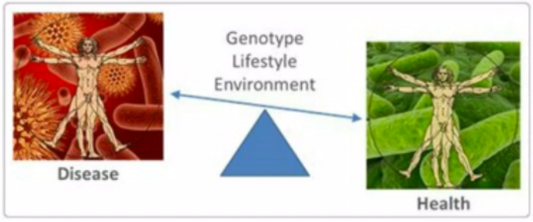
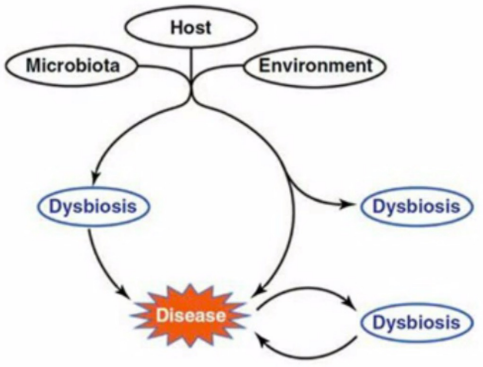
Dysbiosis - cause or consequence of disease?



- 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



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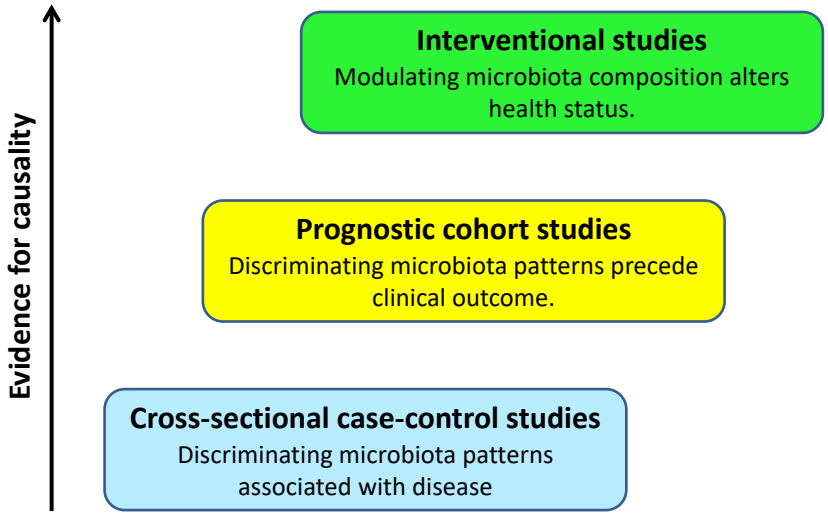
Study design defines the ability to determine causality



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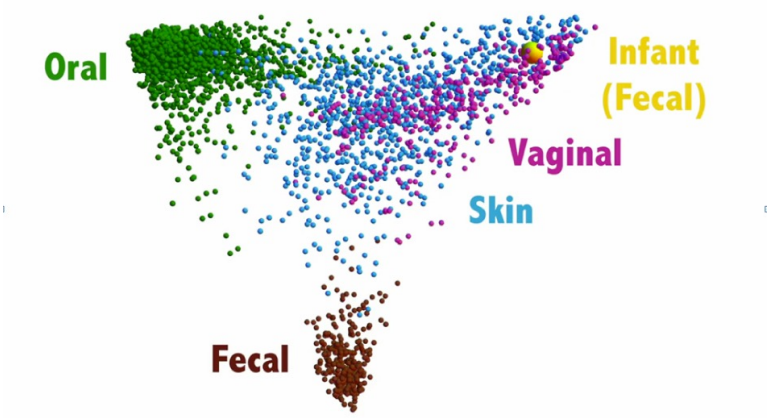
Gut microbiota maturation during first 2 years of life



How to avoid deleterious effect of antibiotics on gut microbiota, while preserving systemic anti-microbial activity?

Watch video: http://www.immulab.fr/cms/index.php/teaching/immunology-teaching

Dr. Rob Knight

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Avoid intestinal effect of antibiotics

- Antibiotics (yellow) 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) released in the colon absorbs/inactivates 99% of antibiotics.
- DAV132 retains normal antibiotic plasma levels, but reduces fecal levels and protects gut microbiota from antibiotic induced dysbiosis.

Watch video: http://www.immulab.fr/cms/index.php/teaching/immunology-teaching

De Gunzburg *et al* JCI 2017

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Take home message

- Gut microbiota influence host immunity (Tolerance versus inflammation)
- 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 is insufficiently schooled to regulate inflammatory responses.
- **Save our microbiota:** Fecal (*C. difficile*) and Vaginal (C-section birth) microbiota transplantation, 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.

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