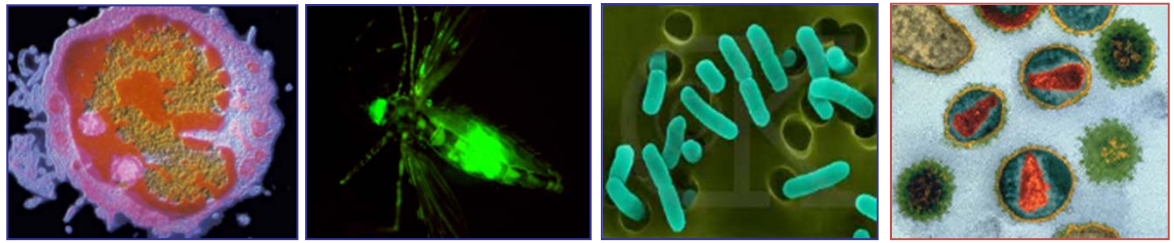


GUT MICROBIOTA, MUCOSAL IMMUNITY AND THEIR IMPACT ON BRAIN PATHOPHYSIOLOGY

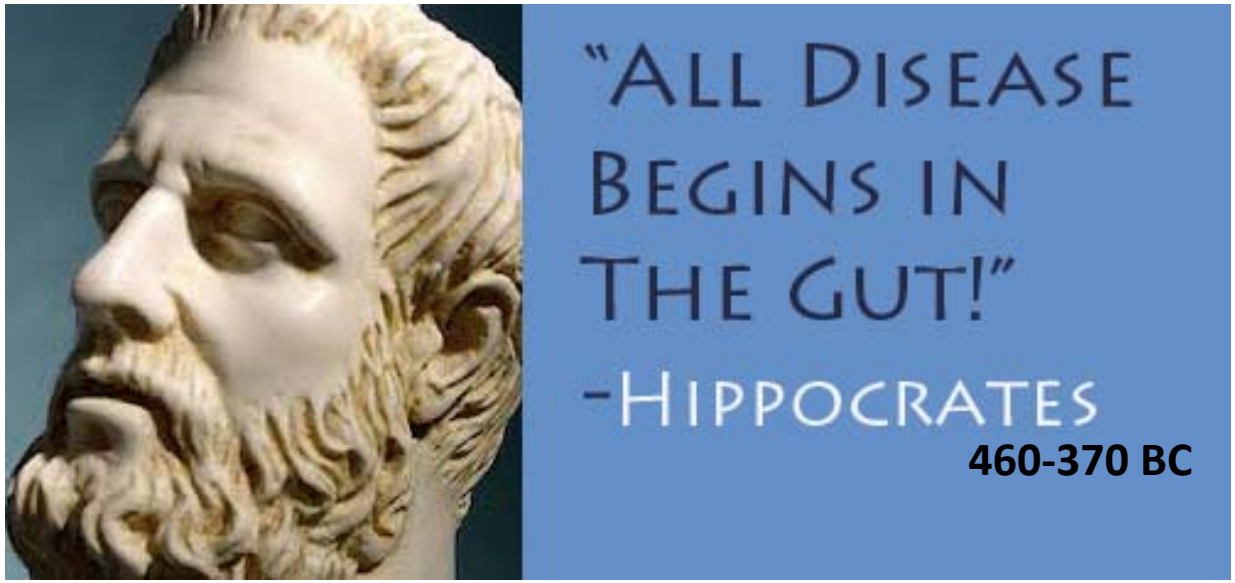
Martin LARSEN

Website: www.immulab.fr
E-mail: Martin.Larsen@upmc.fr

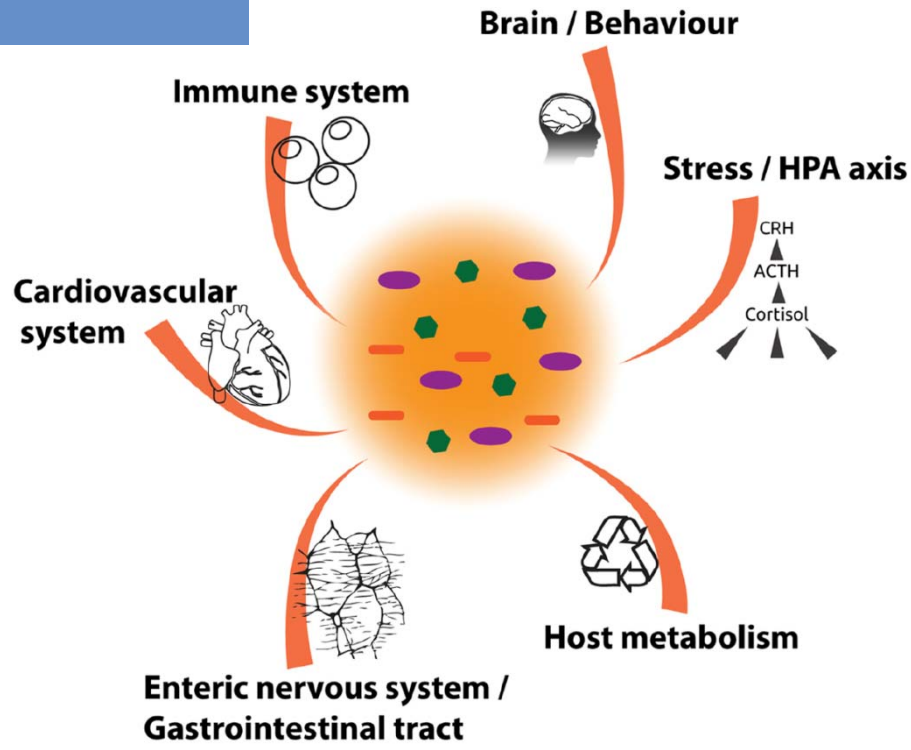
Team : Immunology of Chronic Inflammatory Diseases

INSERM U1135, CHU Pitié-Salpetrière, Paris, France

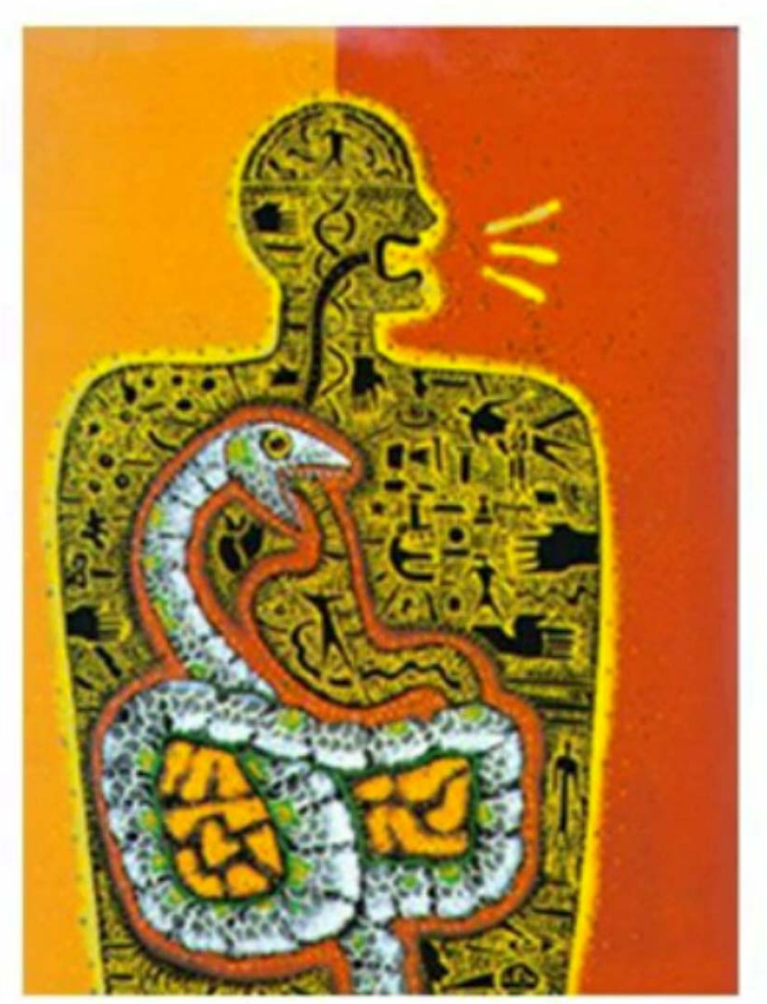
All disease begins in the gut



- First to be recognized for systematically using diet and exercise to treat life-style diseases.

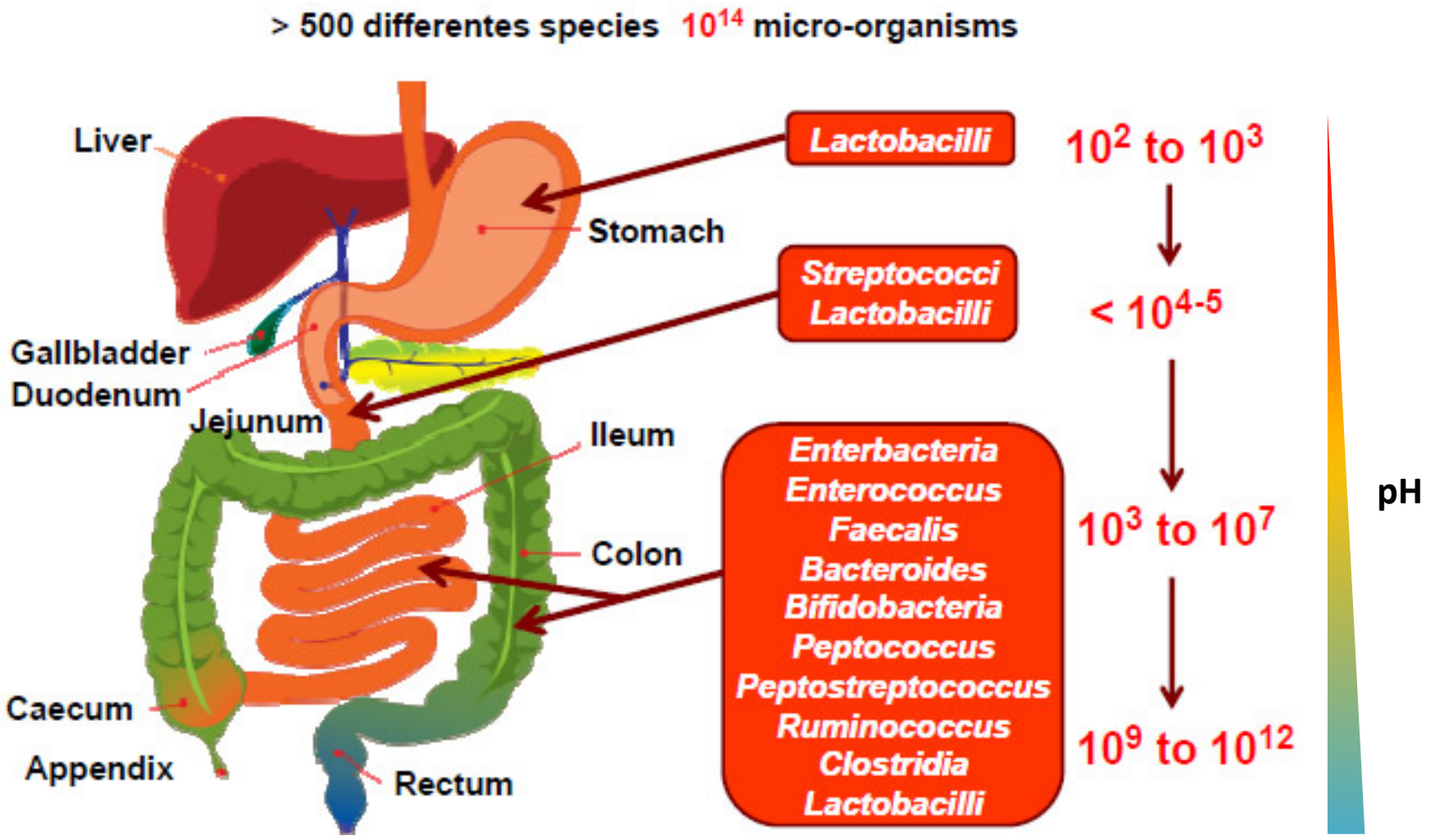


The human Gut and its inhabitants in numbers



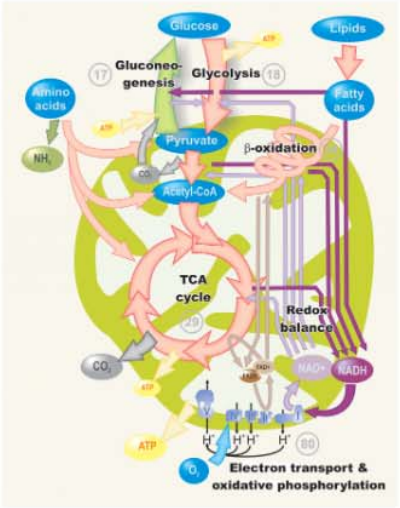
- 30 tons of food and 50.000 L during a lifetime
- Huge mucosal surface:150-200 m^2
- >50 billions of new bacteria every day
- 70-80% of all immune cells are located in the Gut.
- 1-2g secretory IgA per day
- 100 millions of neurons (as many as in the spinal cord).
- 10^{14} bacteria: x10 number of cells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

Spatial distribution of gut microbiota

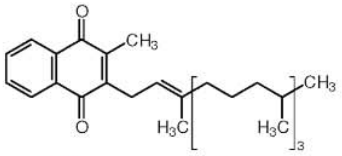


http://www.jpp.krakow.pl/journal/archive/08_15/articles/02_article.html

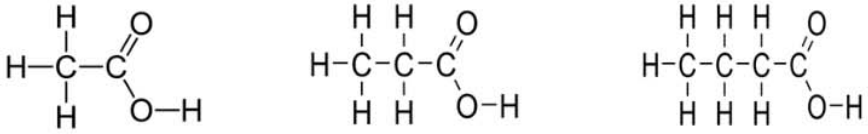
Gut Microbiota in health



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



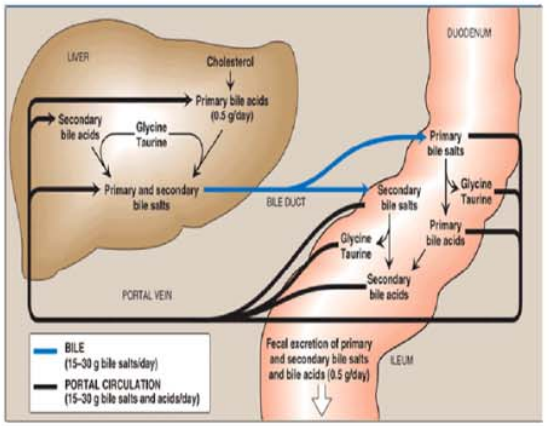
Vitamin synthesis
(eg Vitamin K)

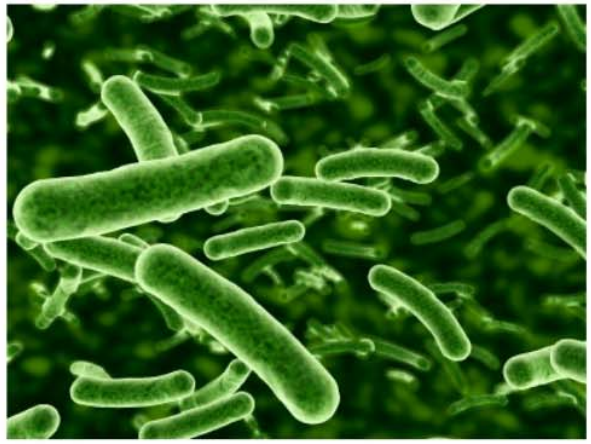


Acetic acid (acetate) Propionic acid (propionate) Butyric acid (butyrate)

Production of short chain fatty acids

Completion of the bile-salt cycle

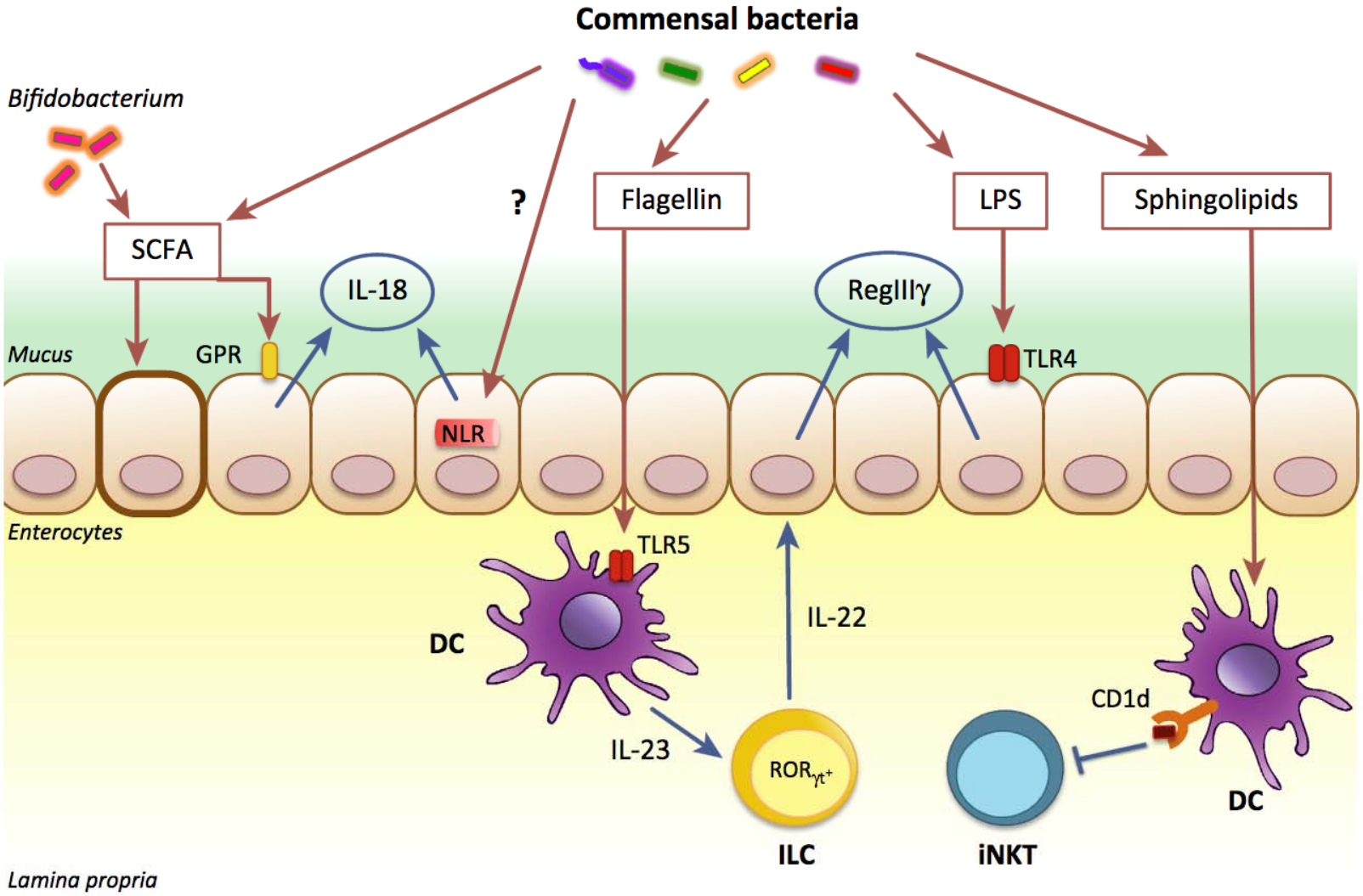




Protect the host from colonization with pathogenic bacteria (Colonization resistance)

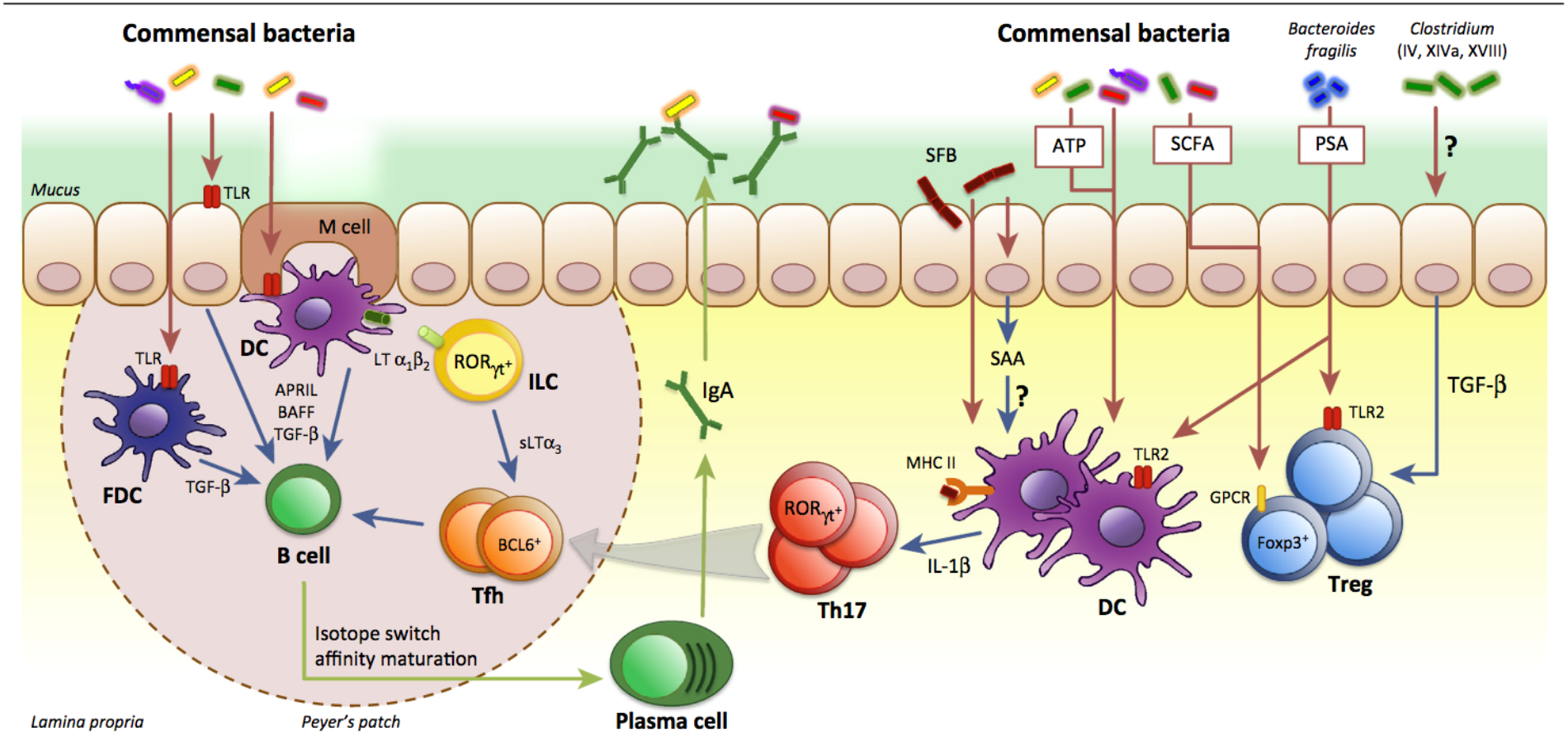
Gut Microbiota in health – Innate immunity

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

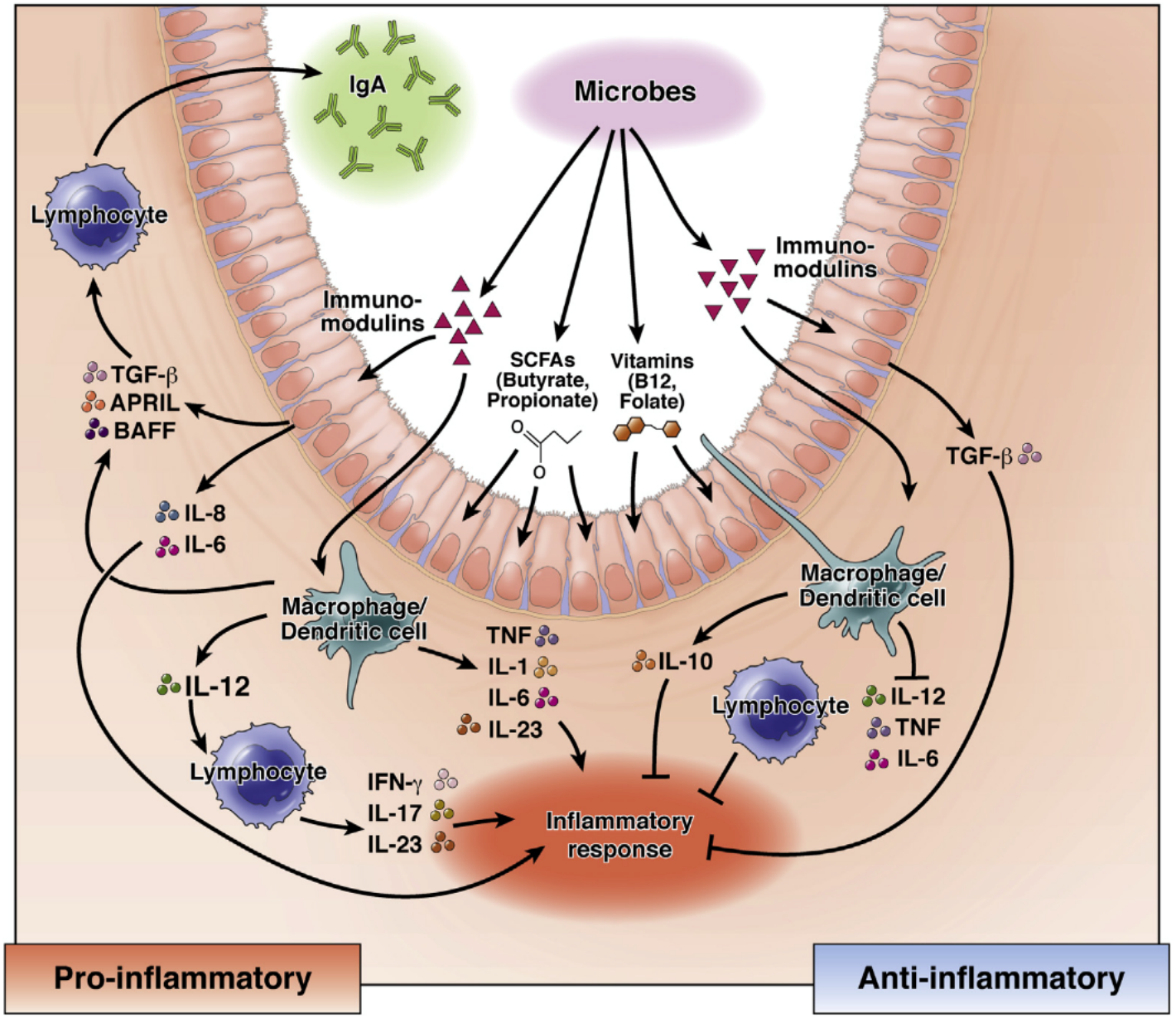


Gut Microbiota in health – adaptive immunity

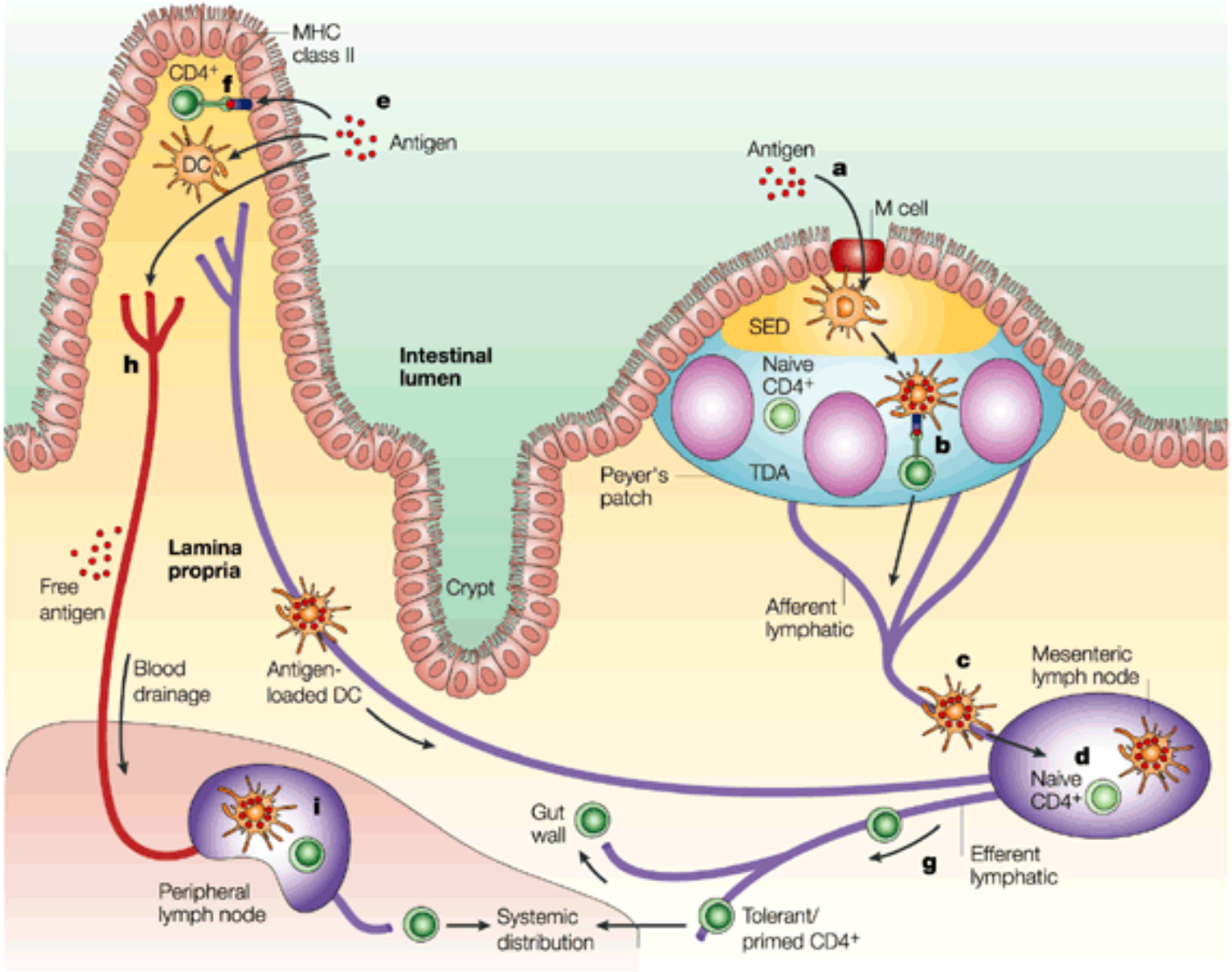
- Microbiota stimulation leads to B cell switch to IgA, regulatory T cell induction, T cell differentiation to Th17



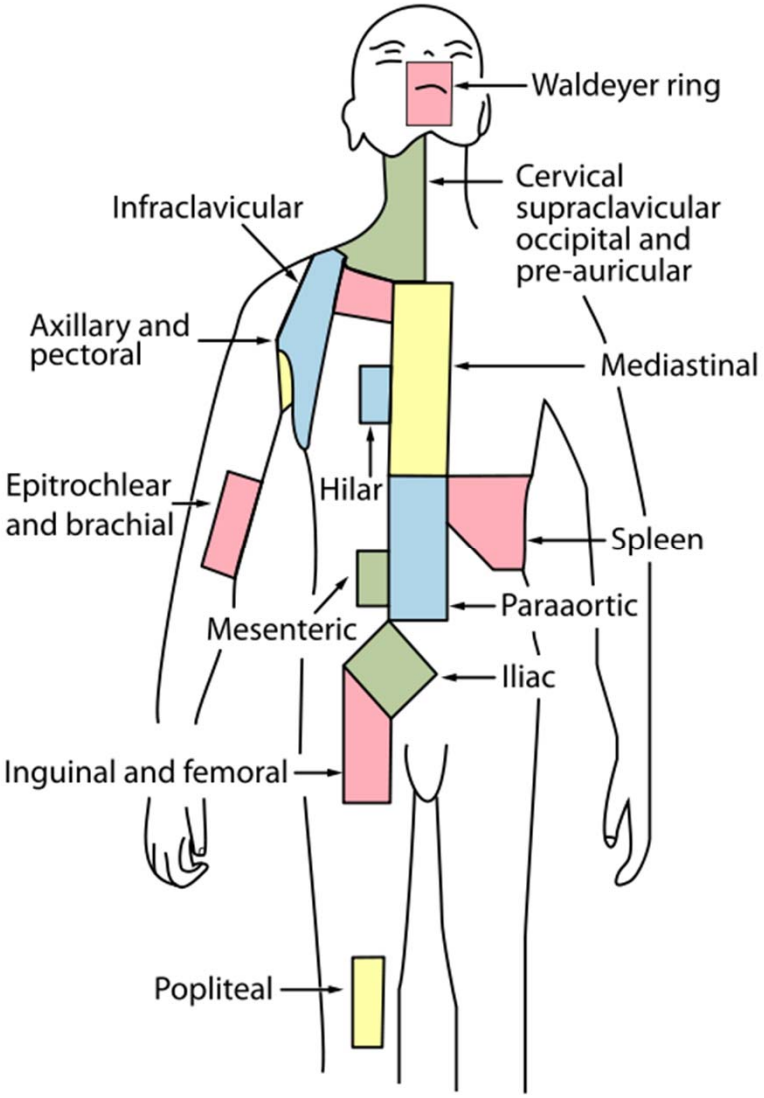
Bacterial-derived Metabolites are key components of cross-talk

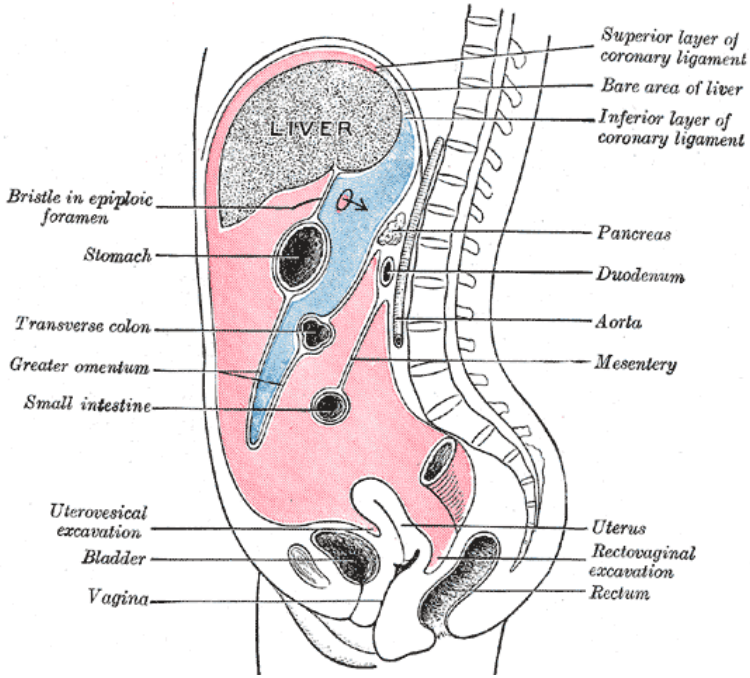


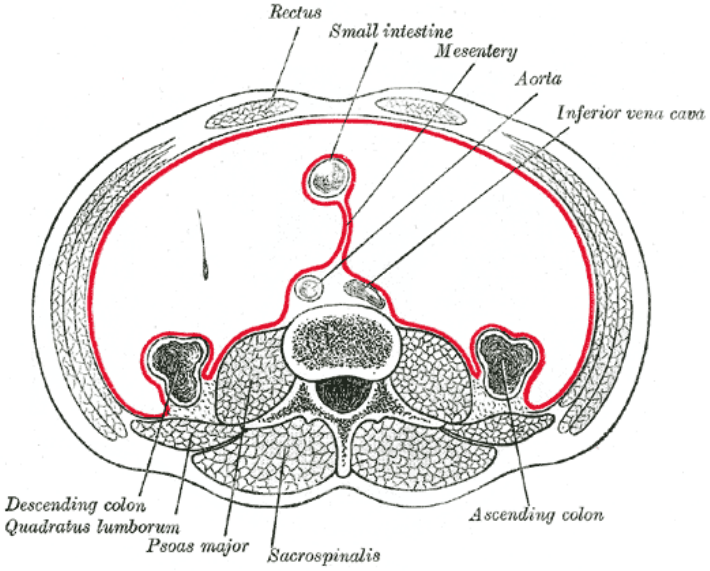
Antibody characteristics



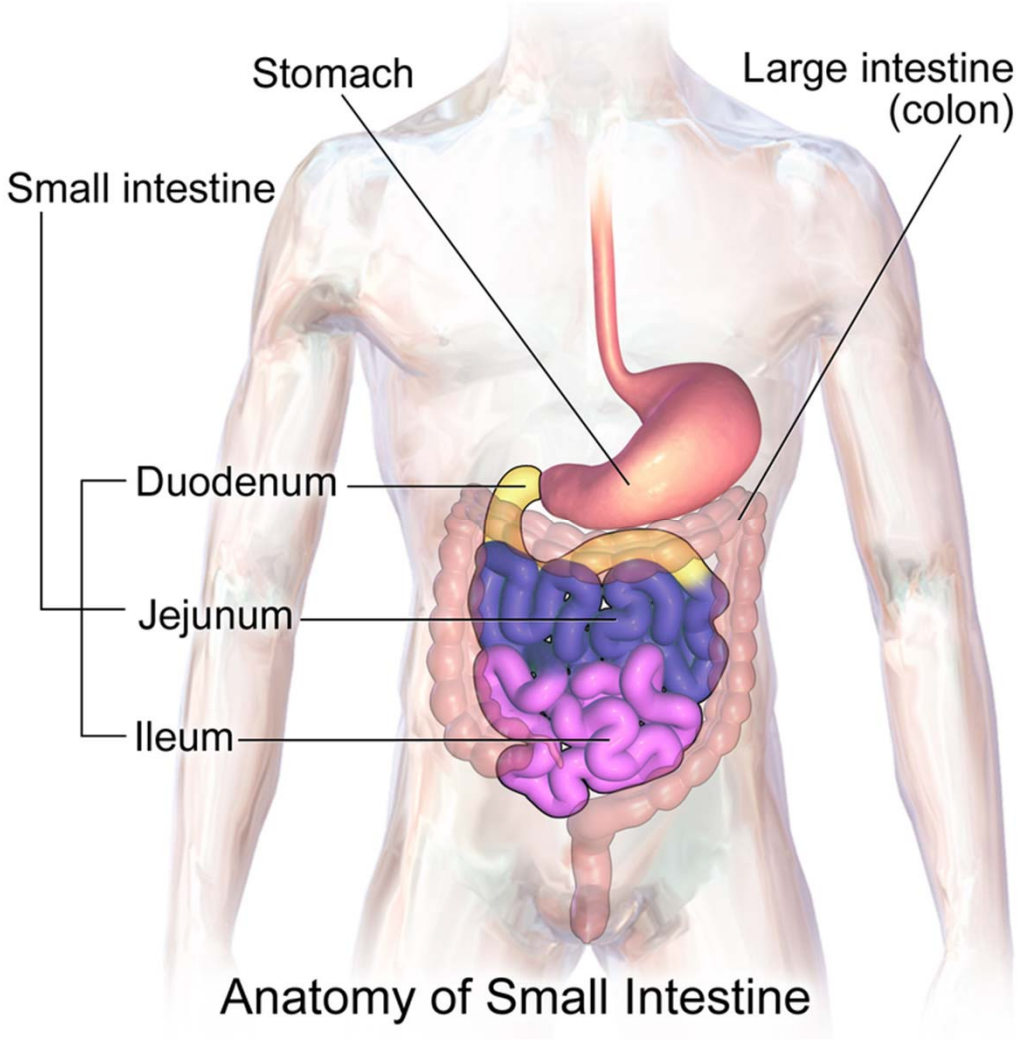
Mesenteric lymph nodes

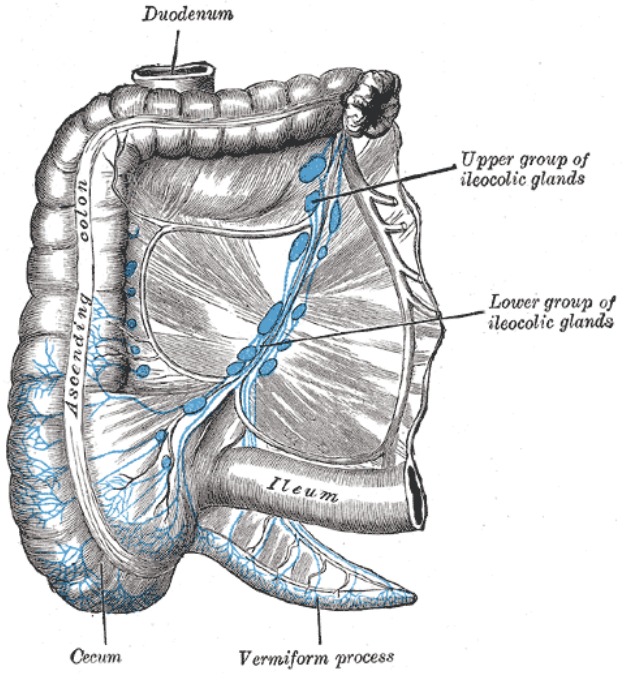


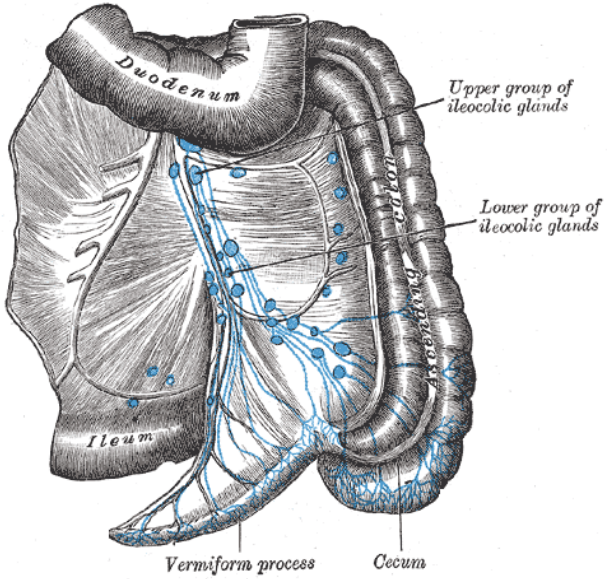




Mesenteric lymph nodes



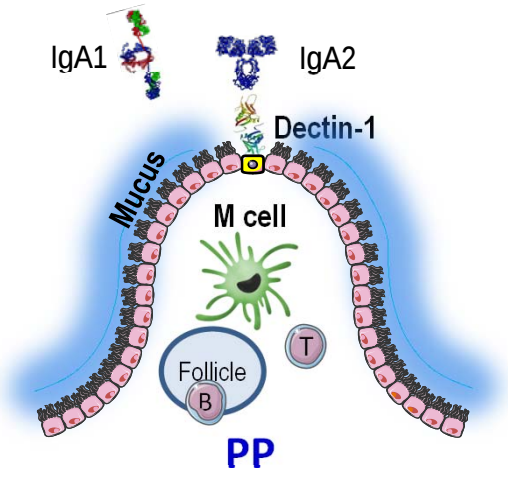


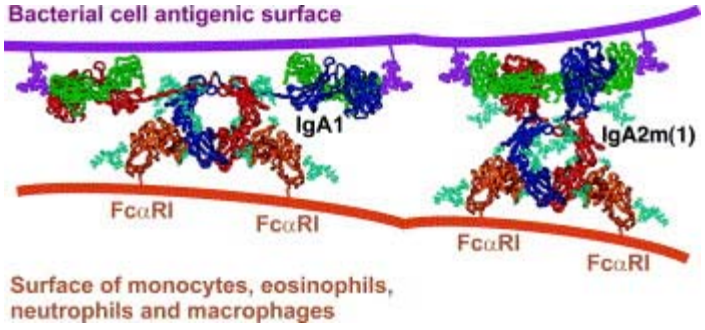


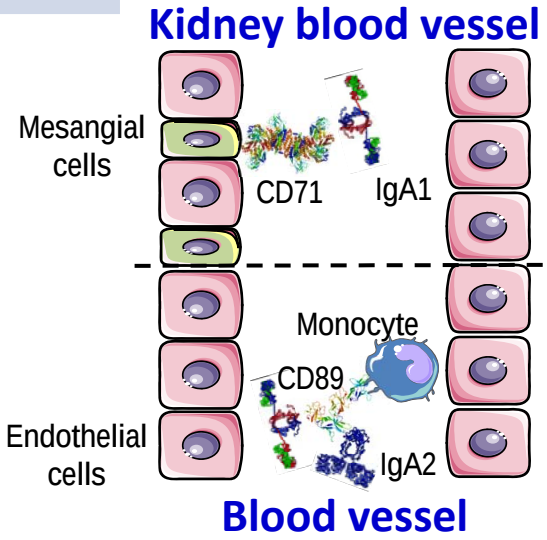
Antibody characteristics

IgA1 IgA2 IgG

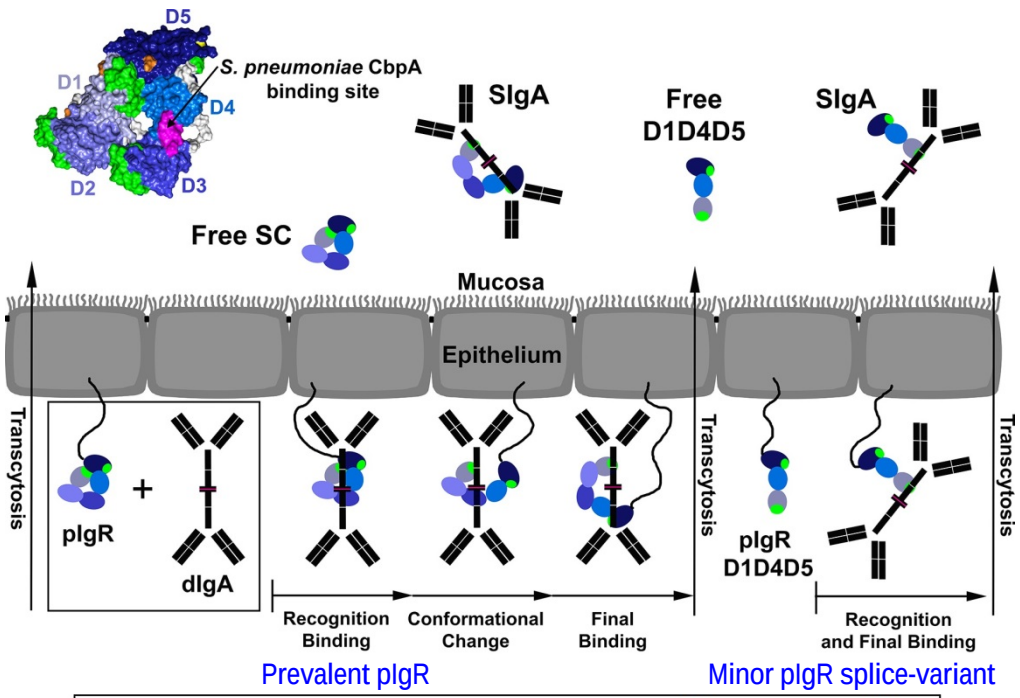
Serum	90%	10%	10mg/ml
Mucosa	50%	50%	0 mg/ml (non-path)
Receptor	pIgR FcaRI (CD89) ASGP-R (liver) CD71 (Mesangial cells, lymphoid cells)	pIgR FcaRI (CD89) ASGP-R (liver) Dectin-1 / Siglec-5 (M cells)	
Protease resistance	Medium	High	Low

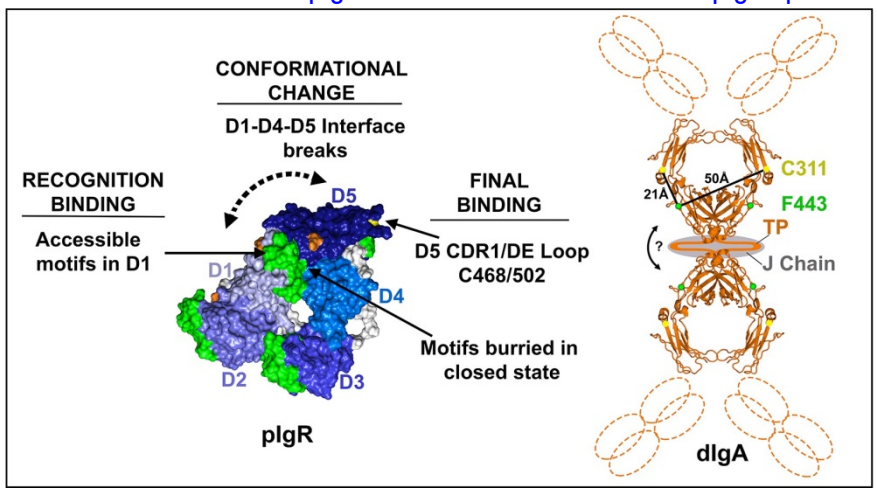






Poly Ig Receptor (pIgR), Secretory component (SC) and pIgs





Human pIgR:

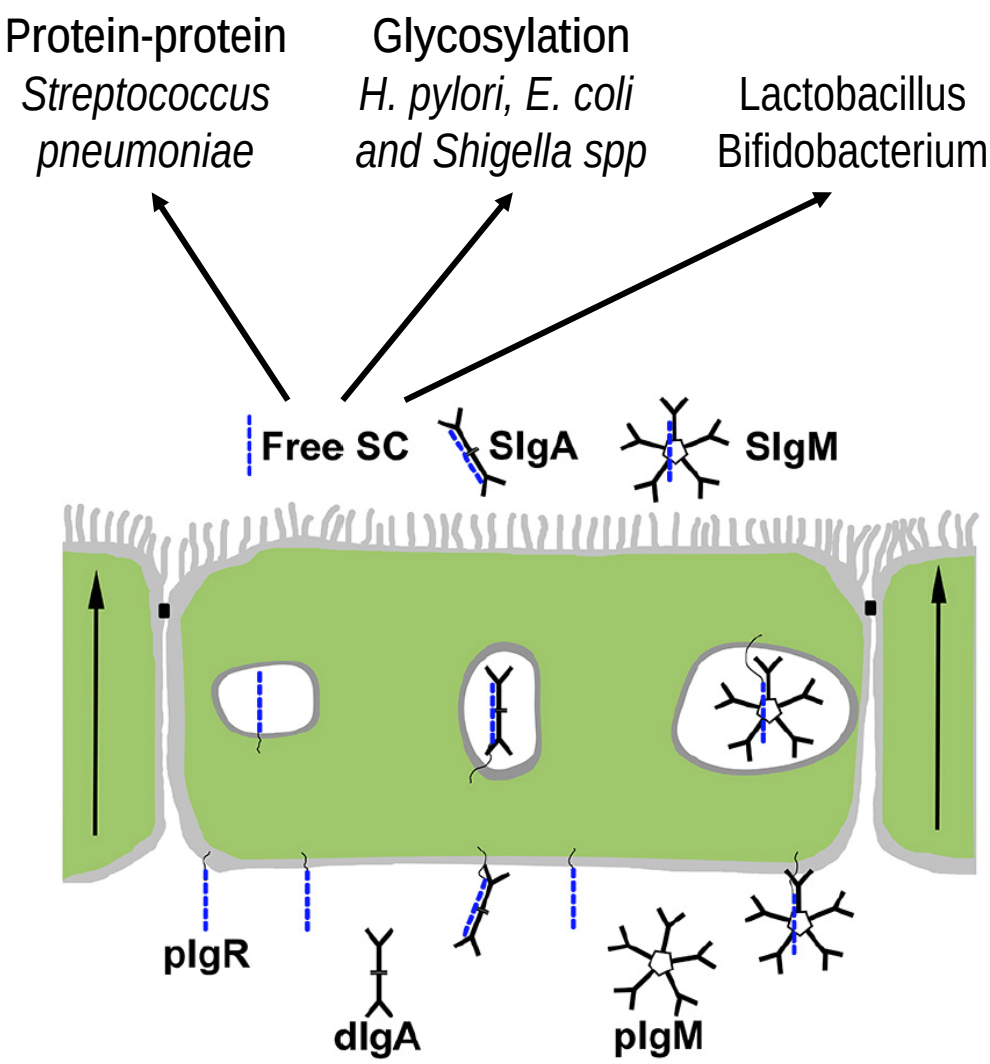
- Glycosylated type I membrane protein
- Five tandem immunoglobulin-like (Ig-like) domains (620AA)
- Transmembrane domain (23AA)
- Intracellular domain (103AA)

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)

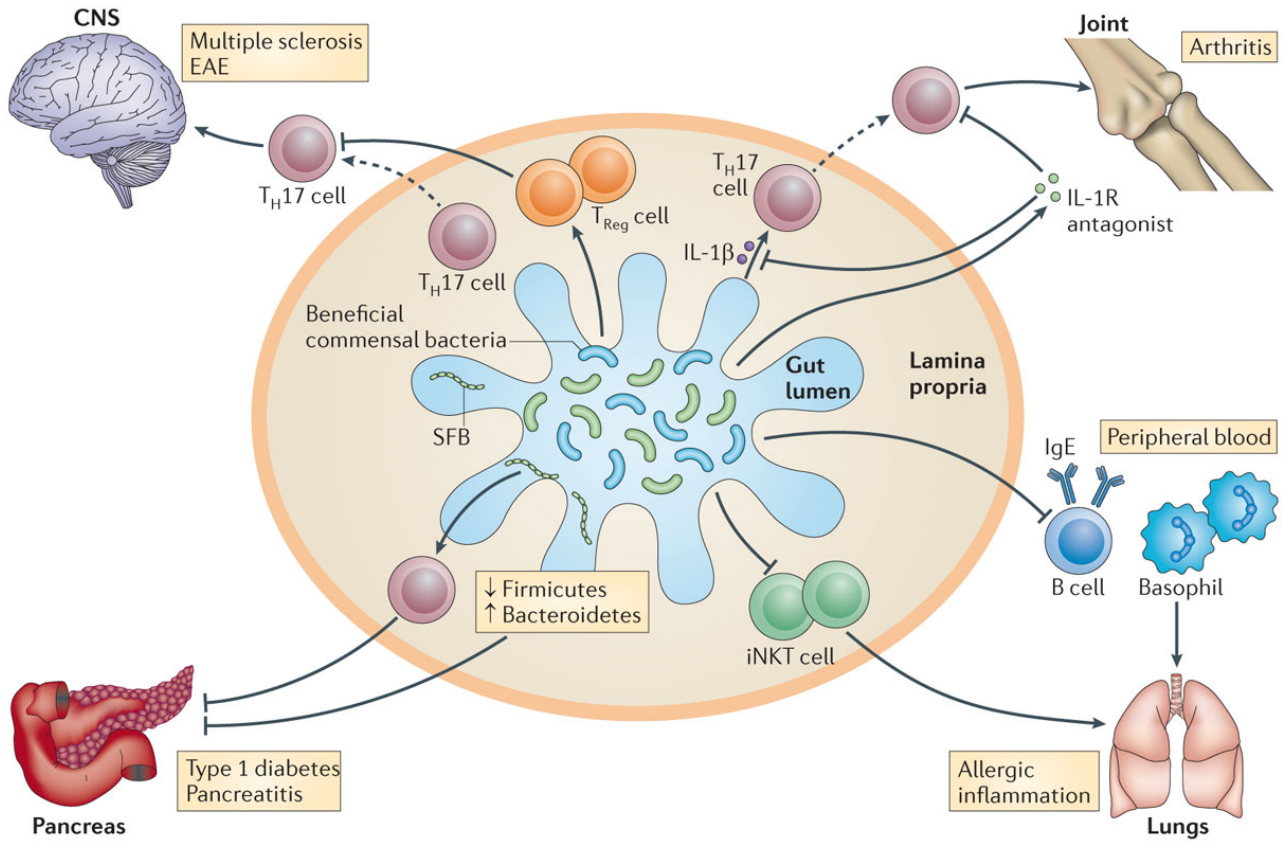
pIgR – pentameric-IgM

- Bound by D1 but not D5.
- Not covalently linked

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

Gut microbiota and autoimmunity

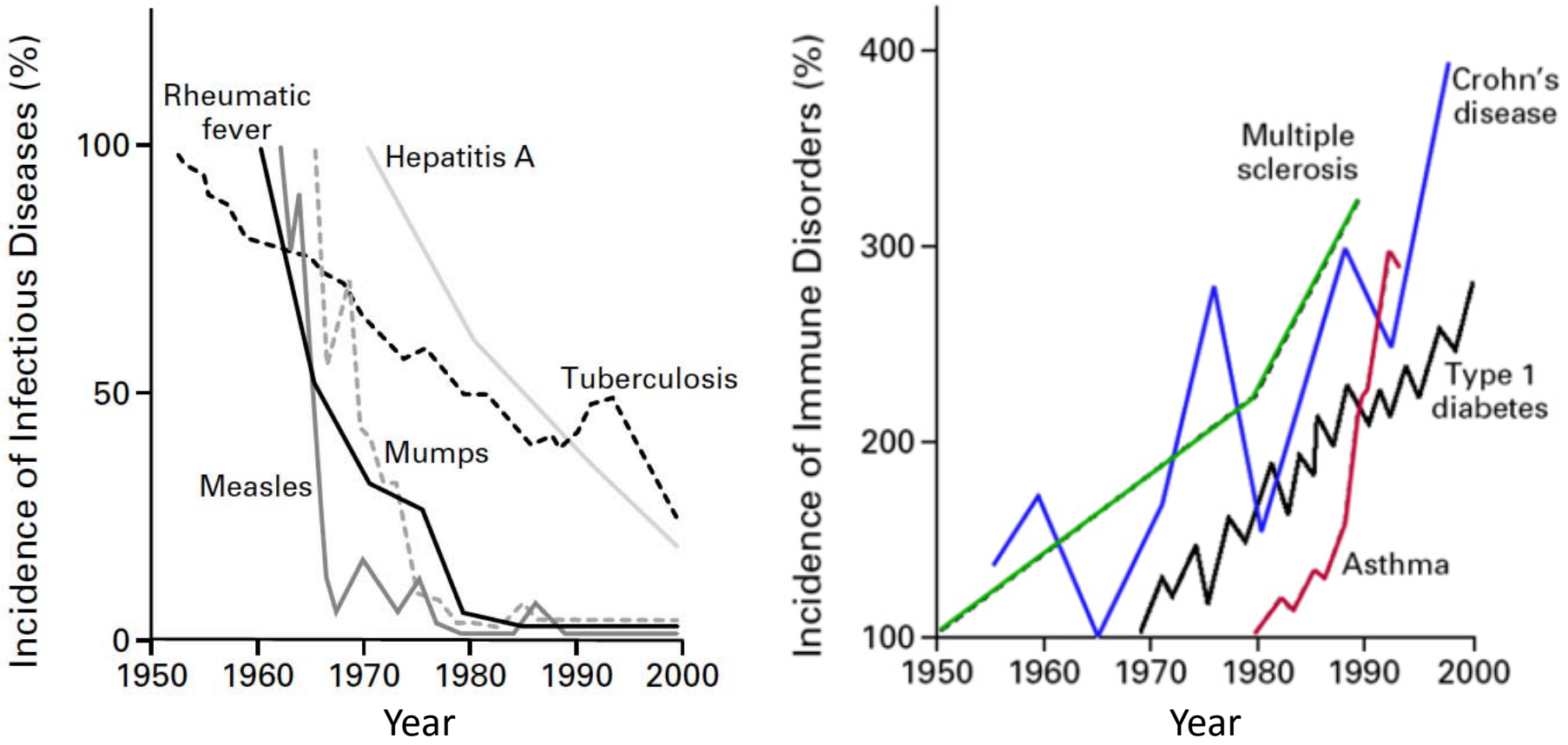
Autoimmunity associated with gut microbiota



- 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

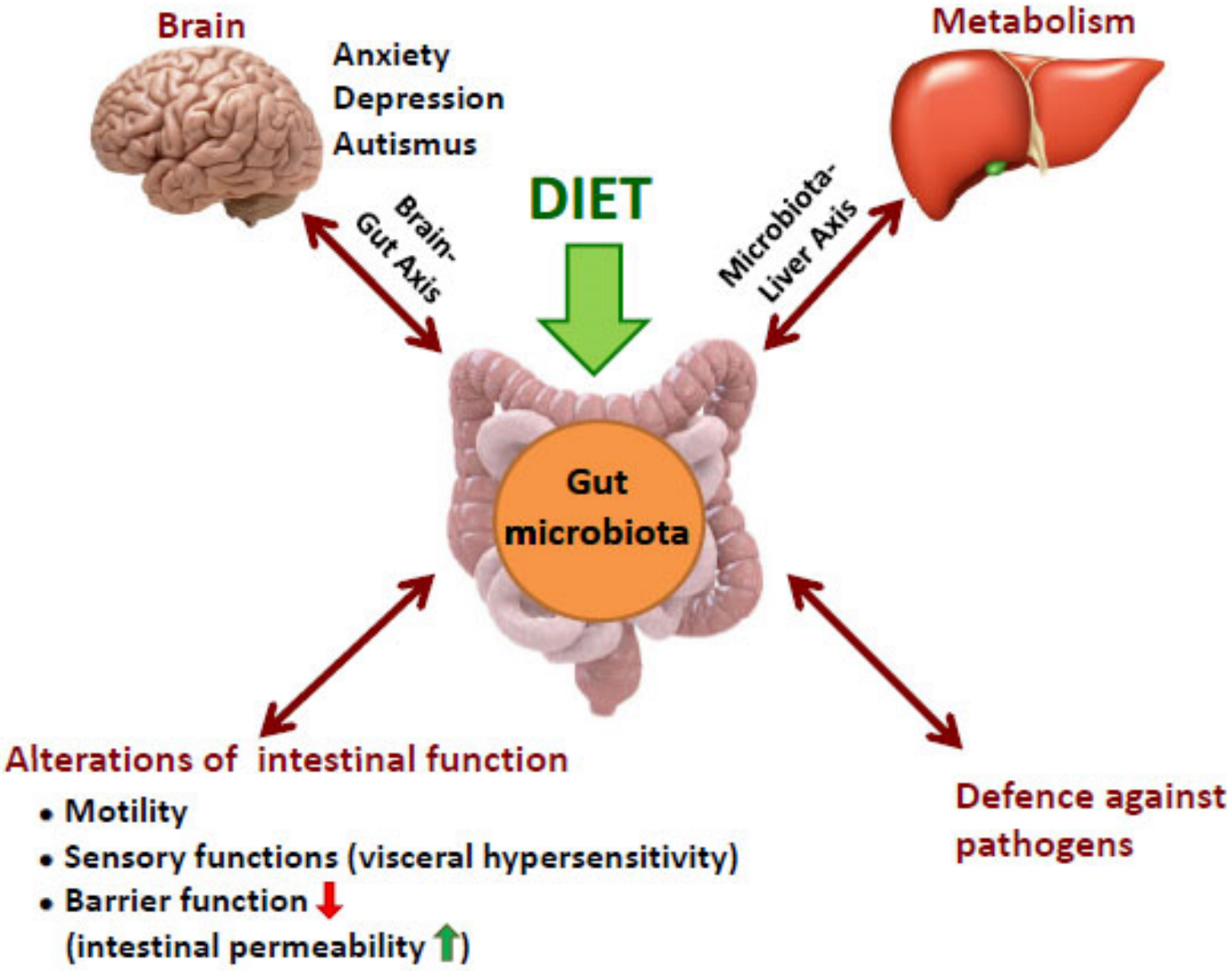
Hygiene theory and autoimmunity



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

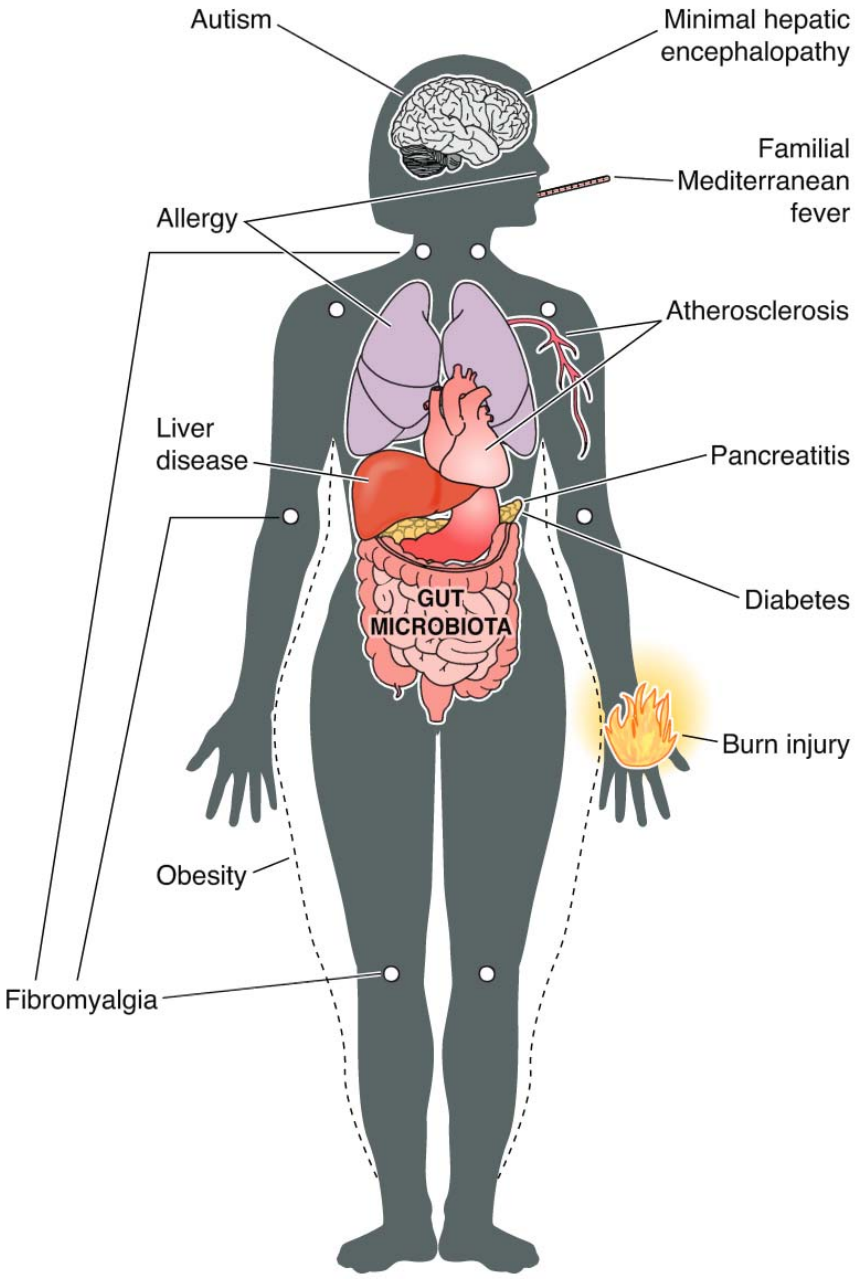
Bach *et al.* NEJM 2002

Gut microbiota – local and distant effects



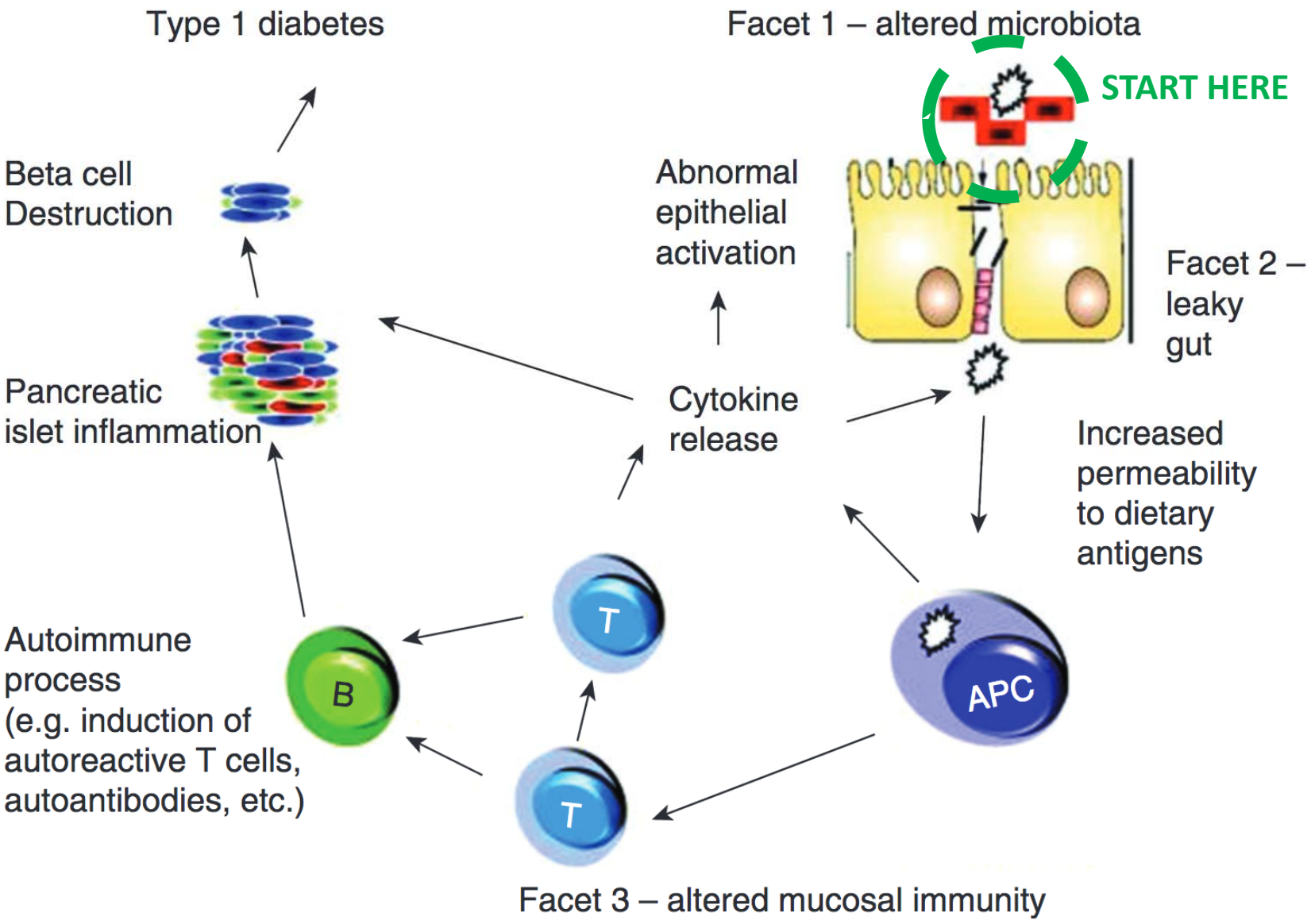
http://www.jpp.krakow.pl/journal/archive/08_15/articles/02_article.html

Gut Microbiota in disease – beyond the gut



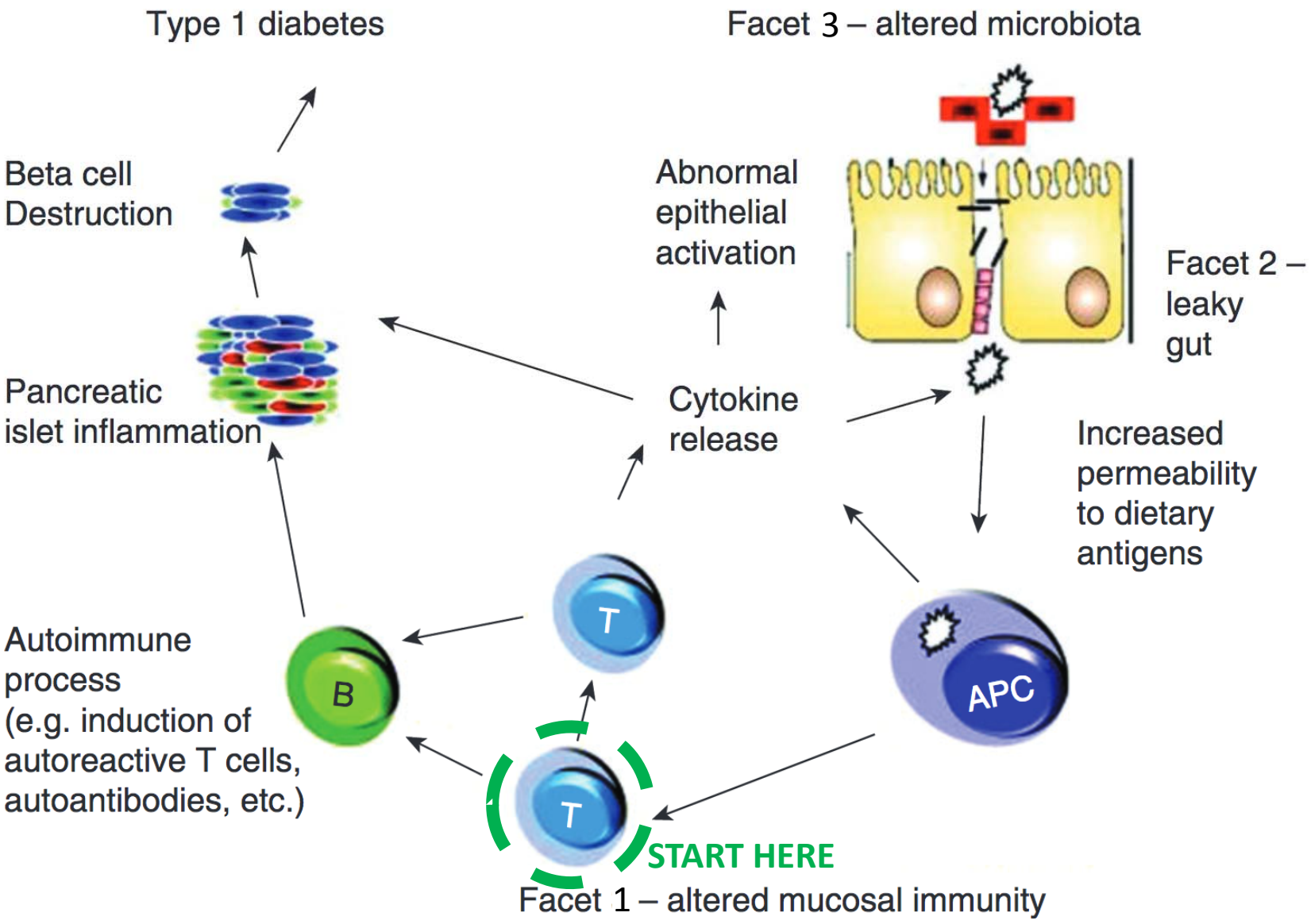
Gut Microbiota – Type-1 diabetes

- Gut leakage - **cause**



Gut Microbiota – Type-1 diabetes

- Gut leakage - **consequence**



Gut Microbiota – Type-2 diabetes

- Insulin-resistance (preceeding T2D) is associated with branched-chain amino acids.

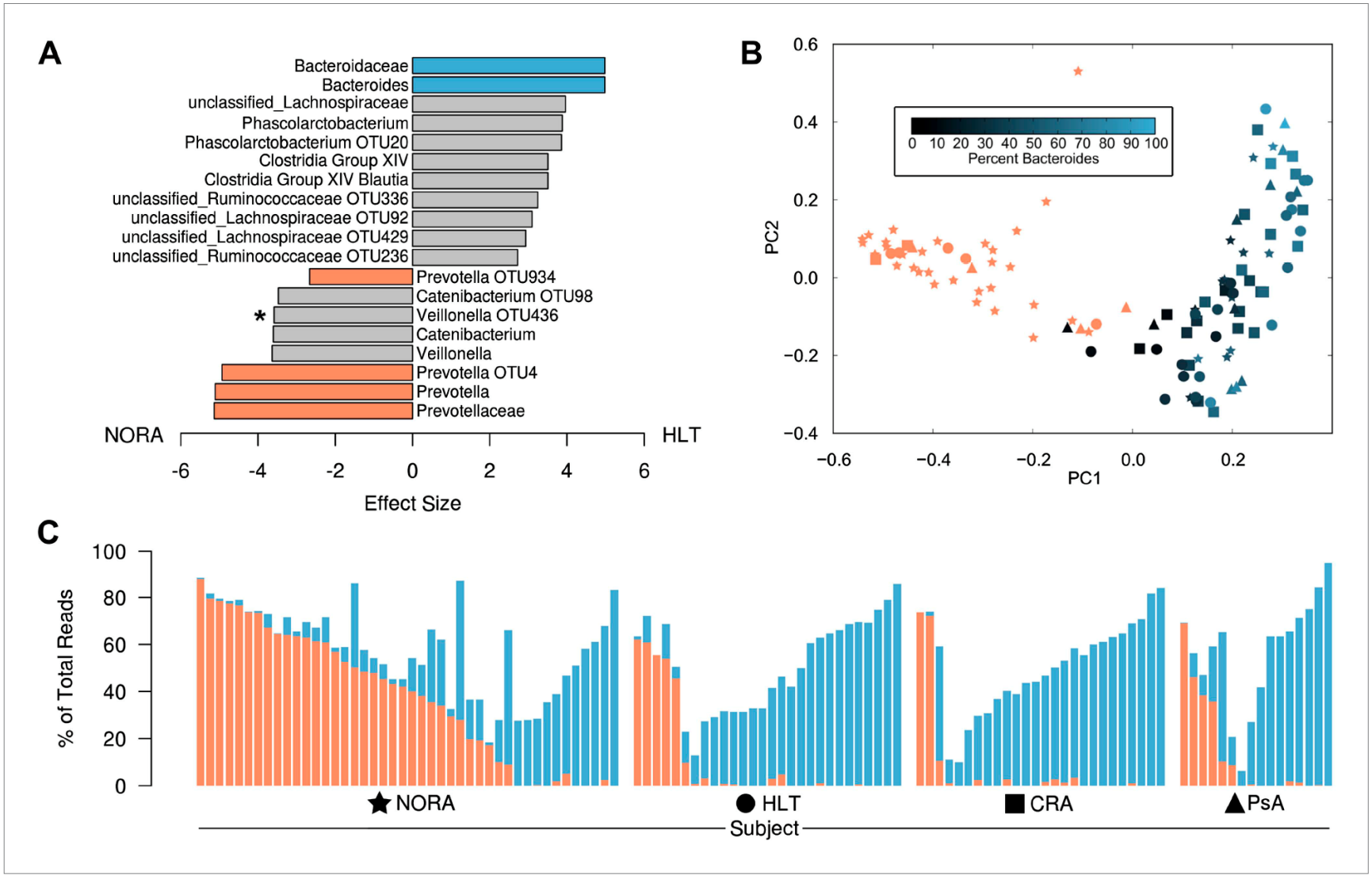
- Prevotella copri can induce insulin-resistance in mice.

  Pedersen et al. Nature 2016

- *E. coli* is increased (treatment bias) and butyrate producers reduced (after controlling for metformin treatment).
  - Qin et al. Nature 2012, Forslund et al. Nature 2015

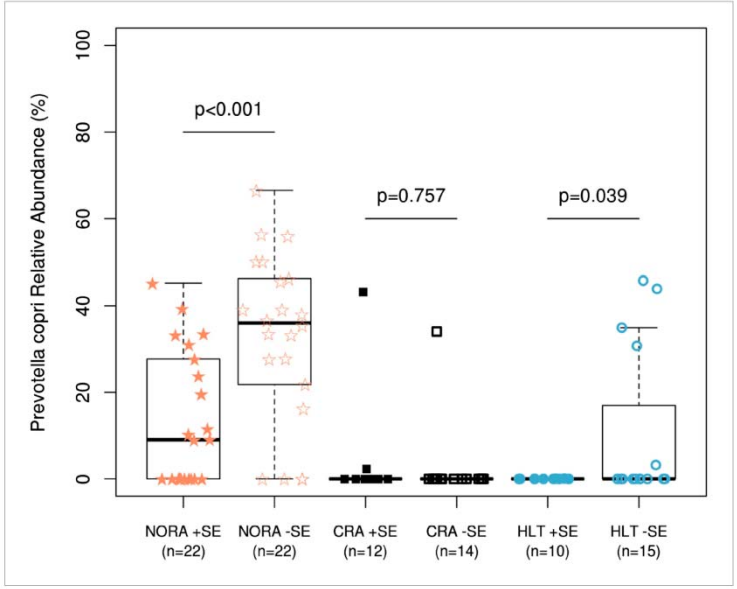
New-Onset Rheumatoid Arthritis - Microbiota

Prevotella Copri is associated with New-Onset Rheumatoid Arthrtis (NORA) but not with chronic RA (CRA) as compared with healthy subjects (HLT).

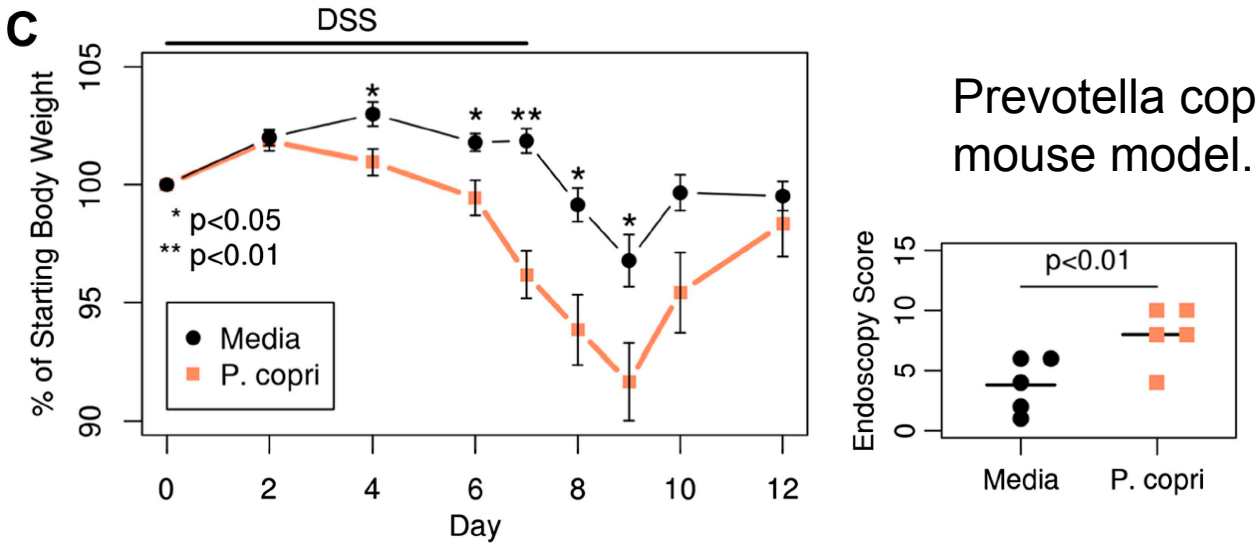


Scher *et al.* eLife 2013

New-Onset Rheumatoid Arthritis - Microbiota



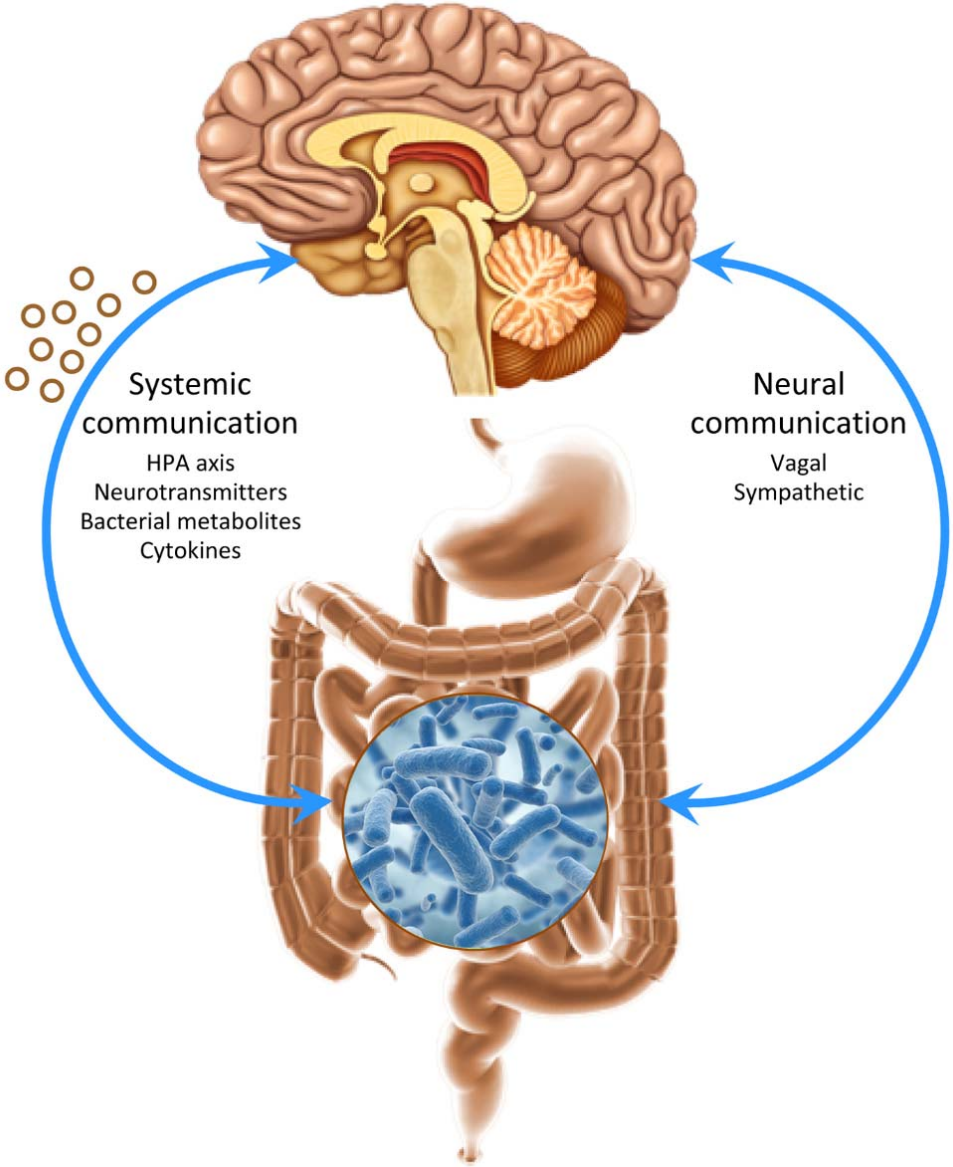
Prevotella Copri is a separate and independent environmental trigger of RA as compared to genetic susceptibility (Shared-epitope risk allele (SE)).



Prevotella copri induce disease in RA mouse model.

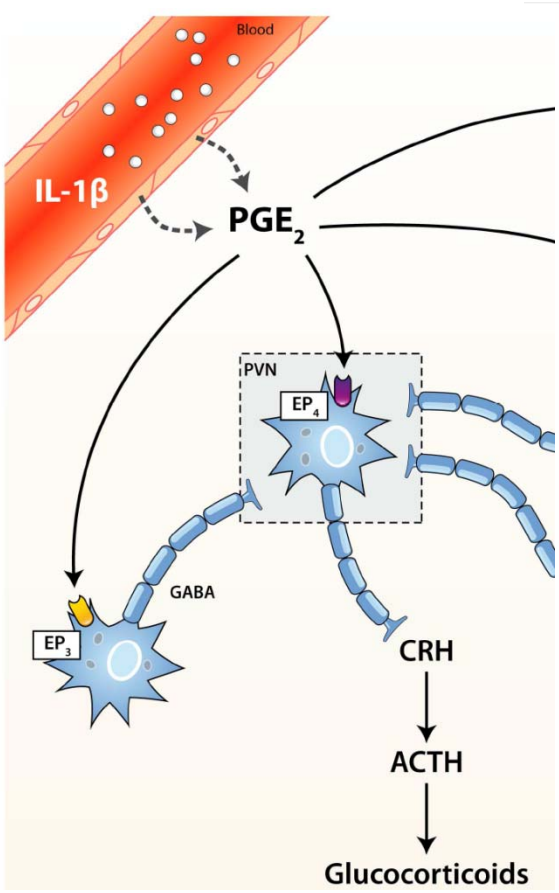
Scher *et al.* eLife 2013

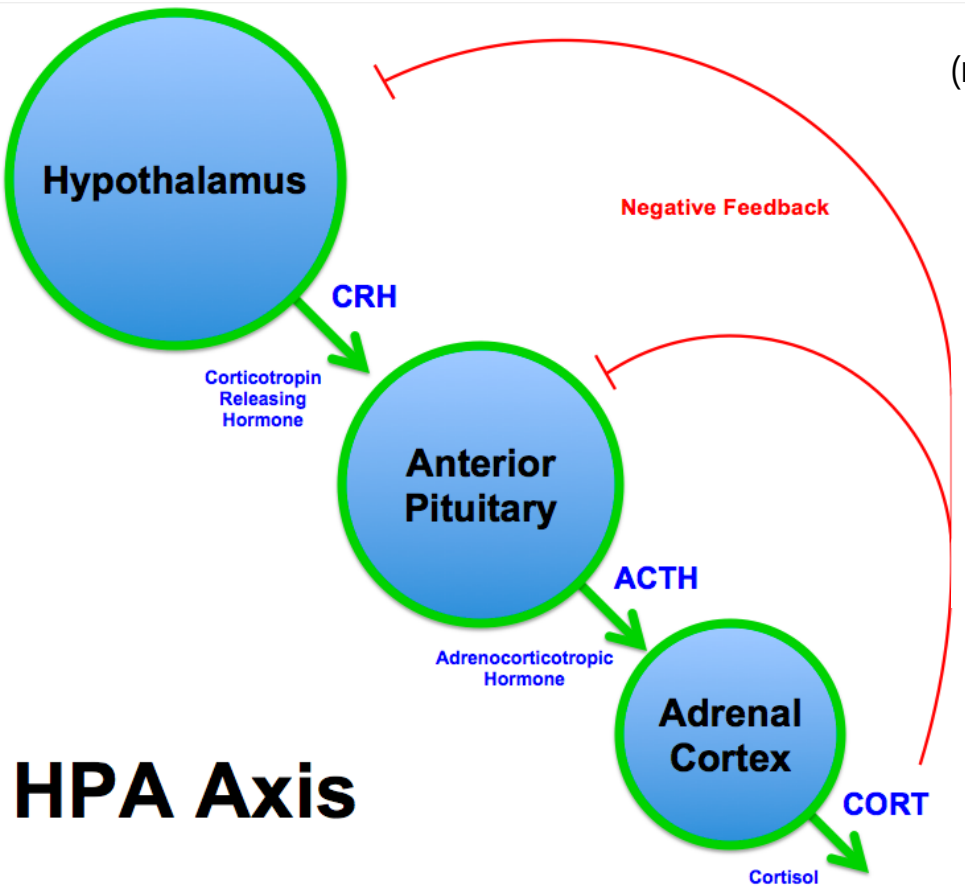
Gut-Brain axis



- Bi-directional communication between gut microbiome, gut, and brain
- Endocrine, neurocrine, and inflammation-related signals from Microbiome and gut can affect brain
- Microbiome may influence stress, anxiety, cognition

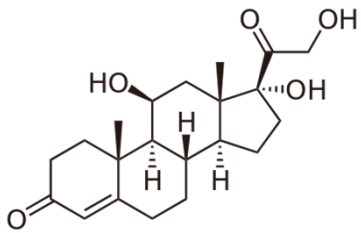
Hypothalamic-pituitary-adrenal (HPA) axis





Proinflammatory
(repress genes controlled by AP-1 & NF-kβ, e.g. TLRs, Il-1β)

Negative feedback



Cortisol

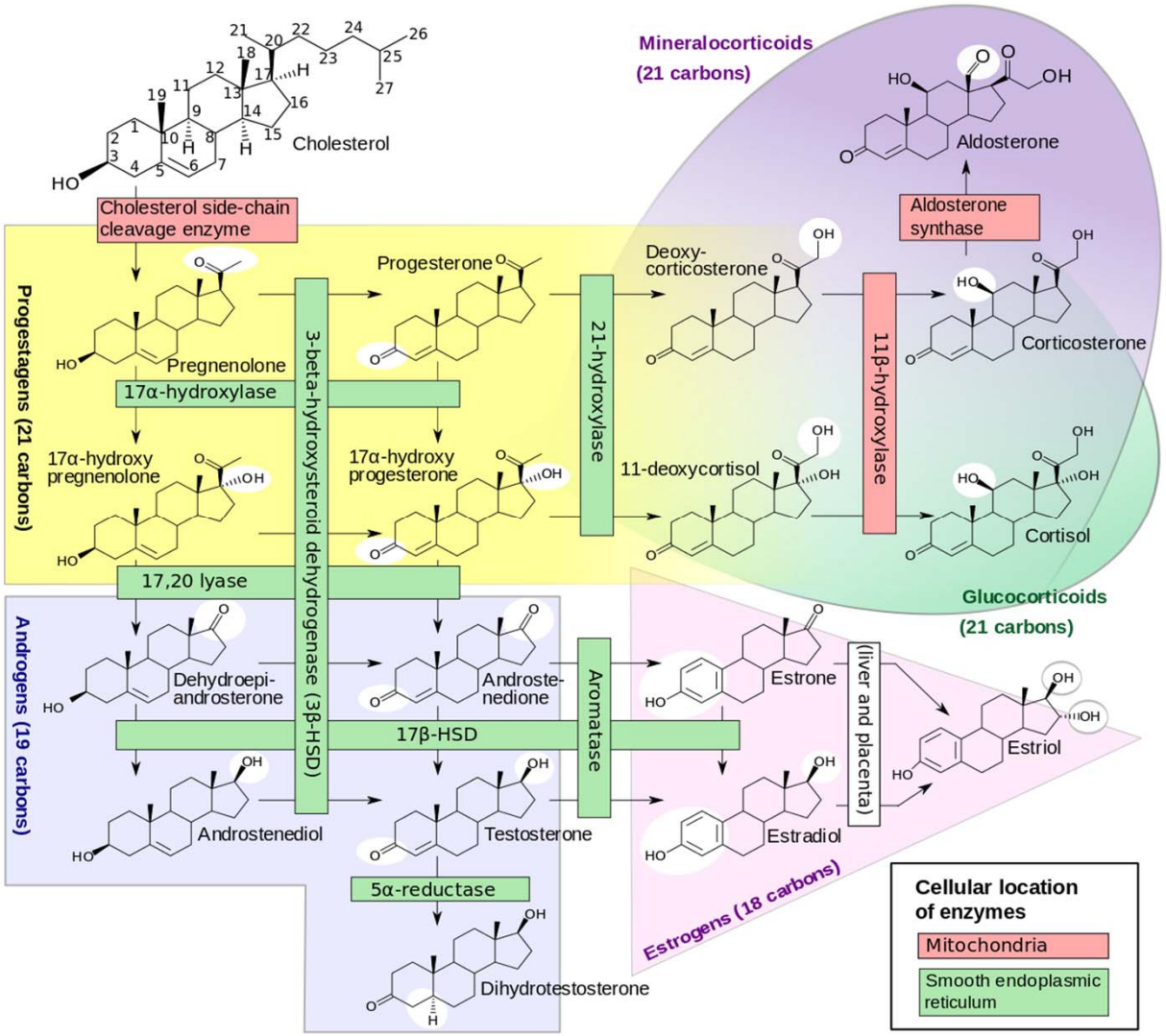
Positive feedback

Anti-inflammatory
(e.g. Il-10, Annexin, MPK-1, GILZ, SOCS)

- Found in all vertebrates (paralleling the evolution of adaptive immunity)
- Cortisol is released in response to immune responses (Il-1β), low blood sugar and stress. Negative feedback of cortisol aims to balance immunity, blood sugar and stress.
- Upregulation of blood sugar is accomplished in part by blocking the high-energy consuming immune system.

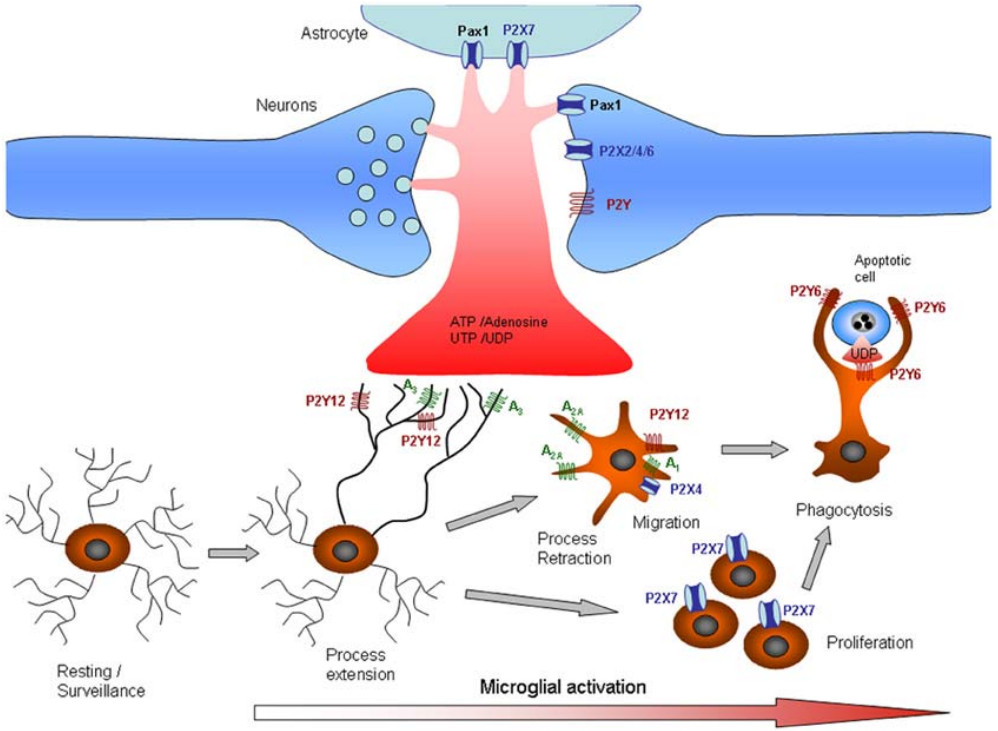
Bellavance et al. Front Immunol 2014

Steroidogenesis – Cholesterol -> Cortisol



- Steroidogenesis: : Glucocorticoids (glucose+cortex+steroid) are synthesized in the cortex from Cholesterol.
- E.g. cortisol (=hydrocortisol)
- Cortisol
  - Increase glucose-level (↑gluconeogenese)
  - Inhibit immune cells
  - Increase metabolism of fat, protein and carbohydrates.
  - Decrease bone formation

Microglia cells – “brain macrophages”

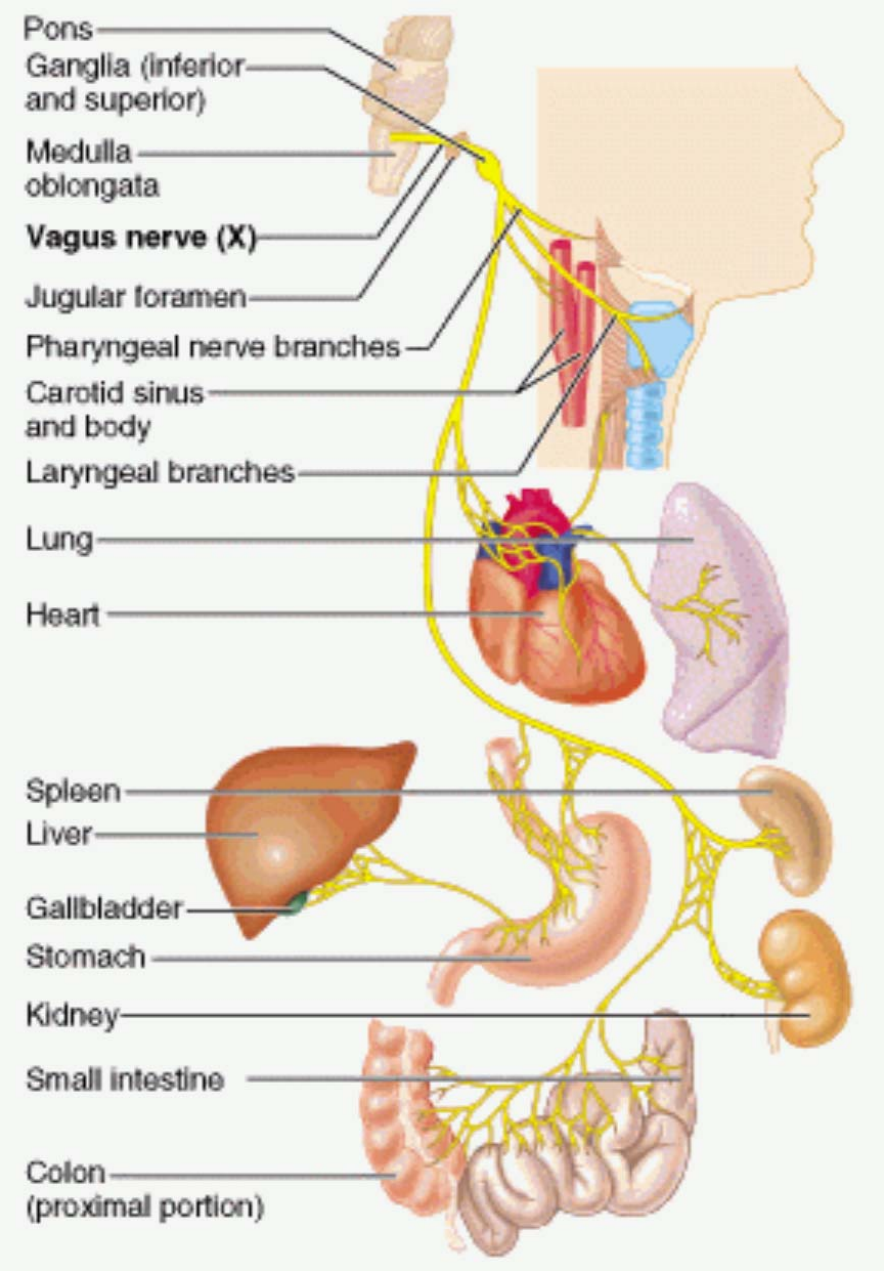


- Microglia cells are derived from hematopoietic stem cells of the bone marrow and resembles (somewhat) monocyte-derived macrophages. Removes “waste”.

- Microglia compose 5-12% of brain cells, has long turn-over (compared to macrophages) and is only recruited from beyond the brain after acute damage to the brain (BBB is transiently rendered permeable to accommodate microglia cells).

- Microglia may be activated by glucocorticoids (GC) through high level of glucocorticoid receptor (GR) expression. These may be derived from the **HPA-axis**.

The vagus nerve



- Brain -> Organ : Vagal efferent
- Organ -> Brain : Vagal afferent

- Acetylcholine is the principle vagal neurotransmitter
  - Reduces cytokine release (TNF-α, IL-1β, IL6 and IL-18) in LPS-stimulated human macrophages.

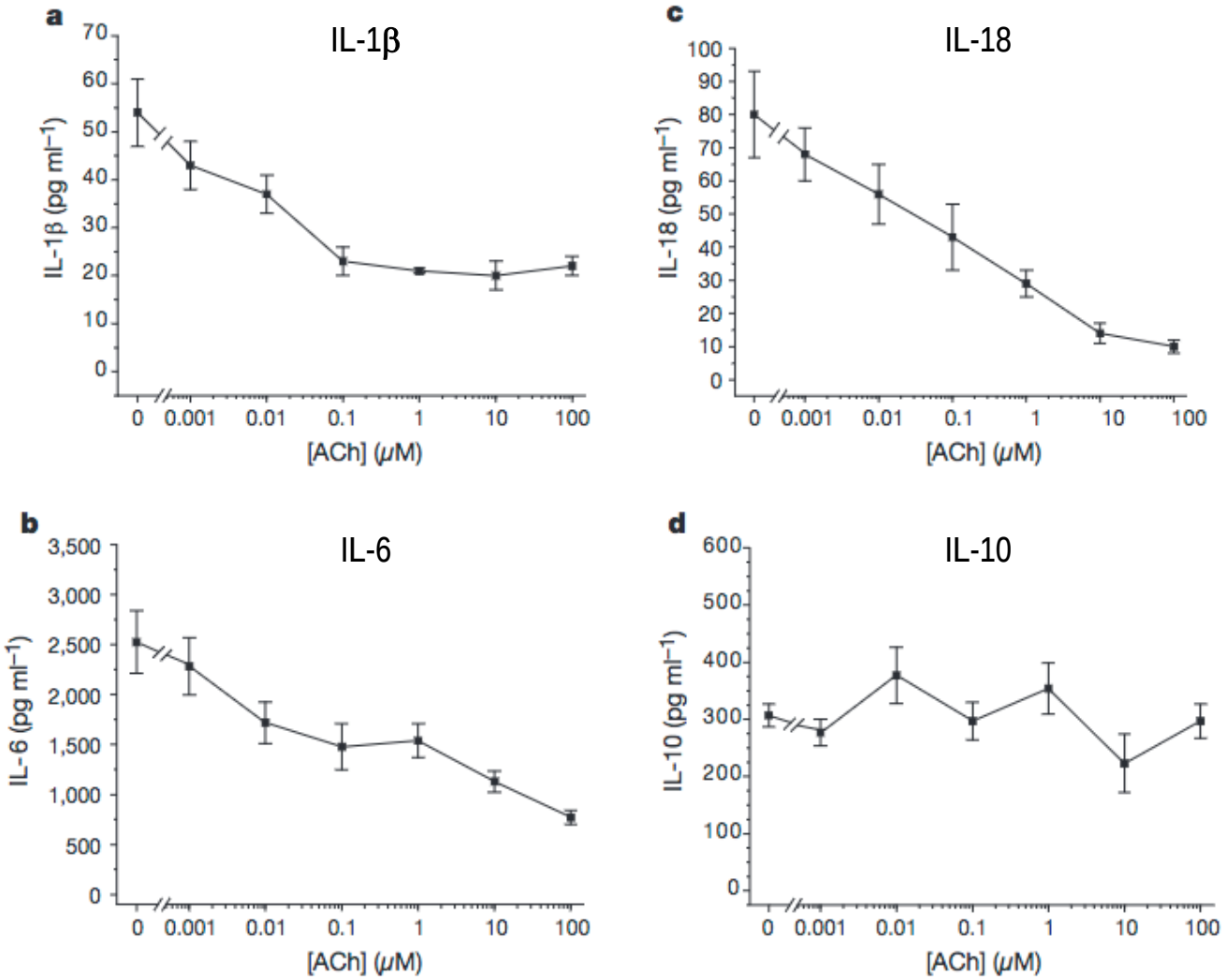
Borovikova et al. Nature 2000

- Insulin indirectly activates vagus nerve, which leads to decreased glucose production by the liver.

- Neuropeptide Y (NPY) partially blocks vagus nerve (anti-anxiety hormone, which stimulate appetite)

- Vagus nerve regulates release of hormones, e.g. oxytocin.
  - Oxytocin release following positive social behaviour.

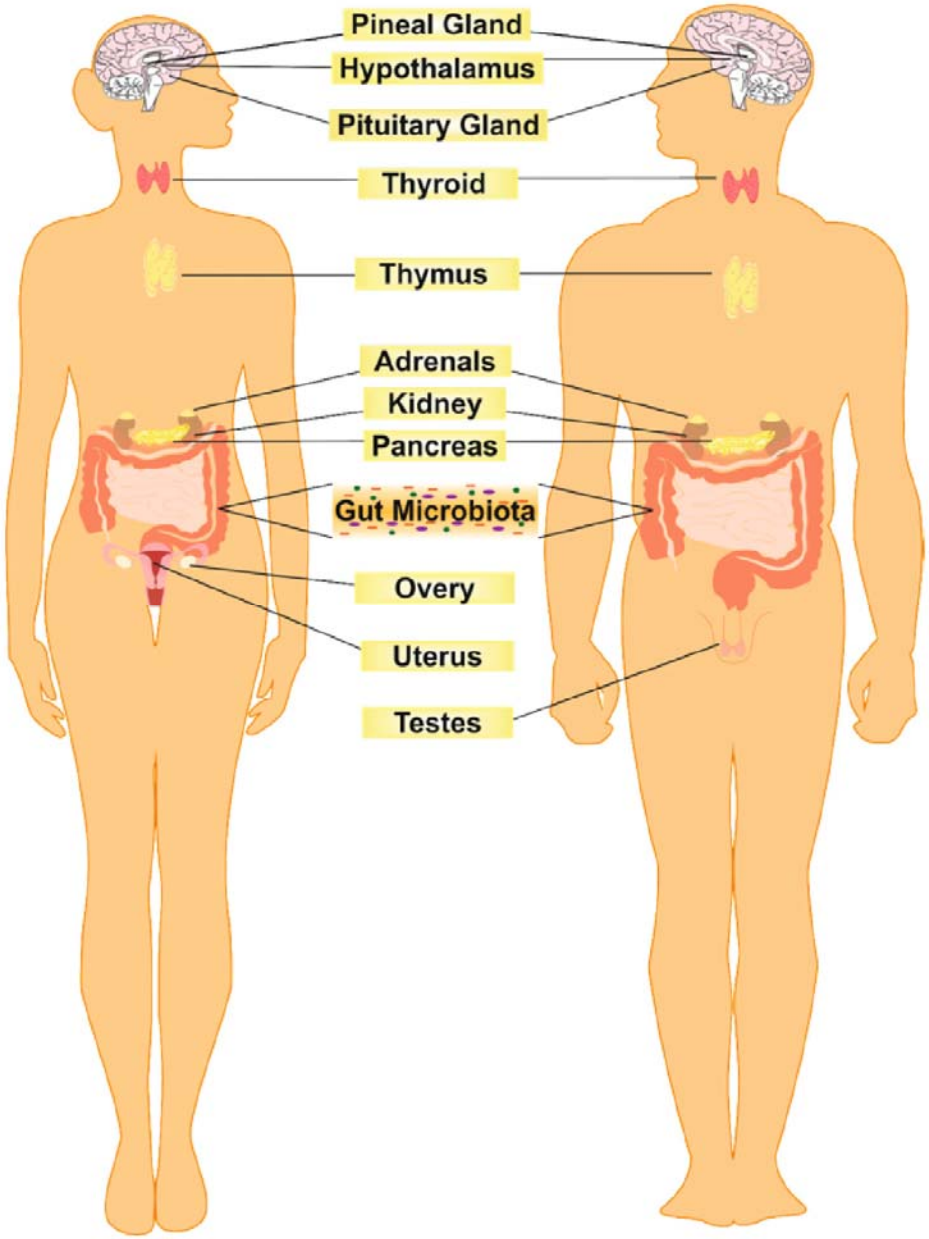
The vagus nerve



- Acetylcholine inhibit LPS-stimulated macrophage secretion of TNF-α, IL-1β, IL-6, IL-18, **BUT NOT** IL-10 (*in vitro*).

Borovikova *et al*. Nature 2000

Gut microbiota has endocrine function

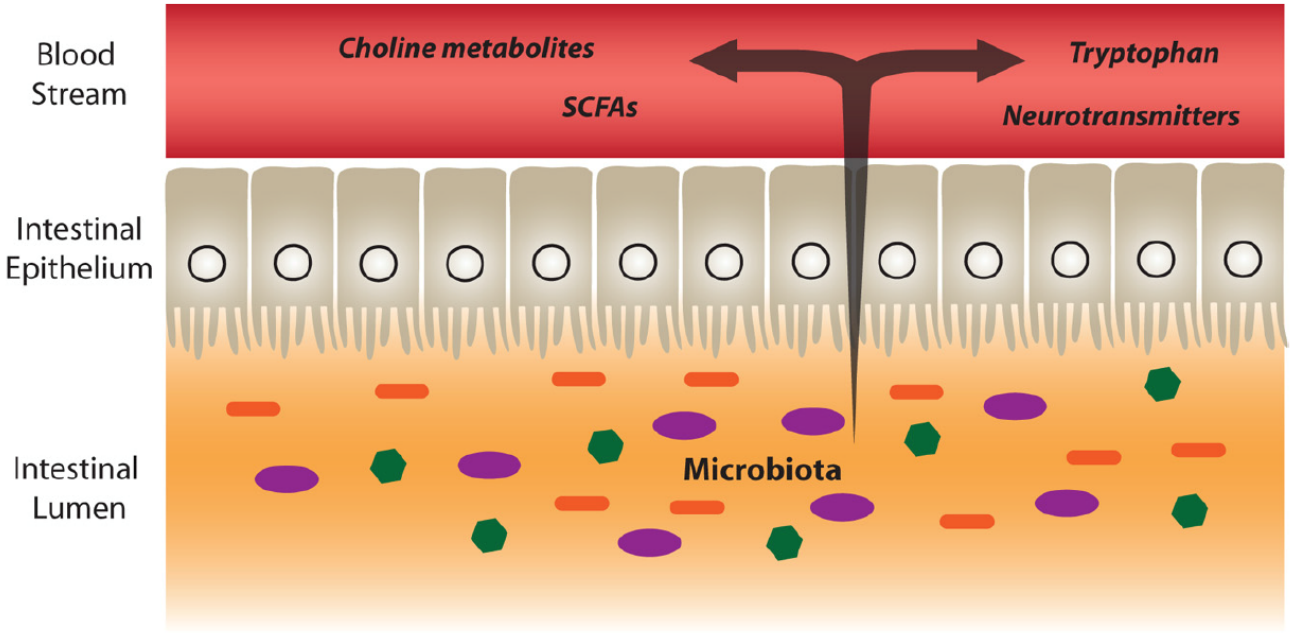


Clarke et al. Mol Endocrin 2014

Gut microbiota has endocrine function

Table 2. Examples of Neurotransmitter-Producing or -Releasing Bacterial Strains

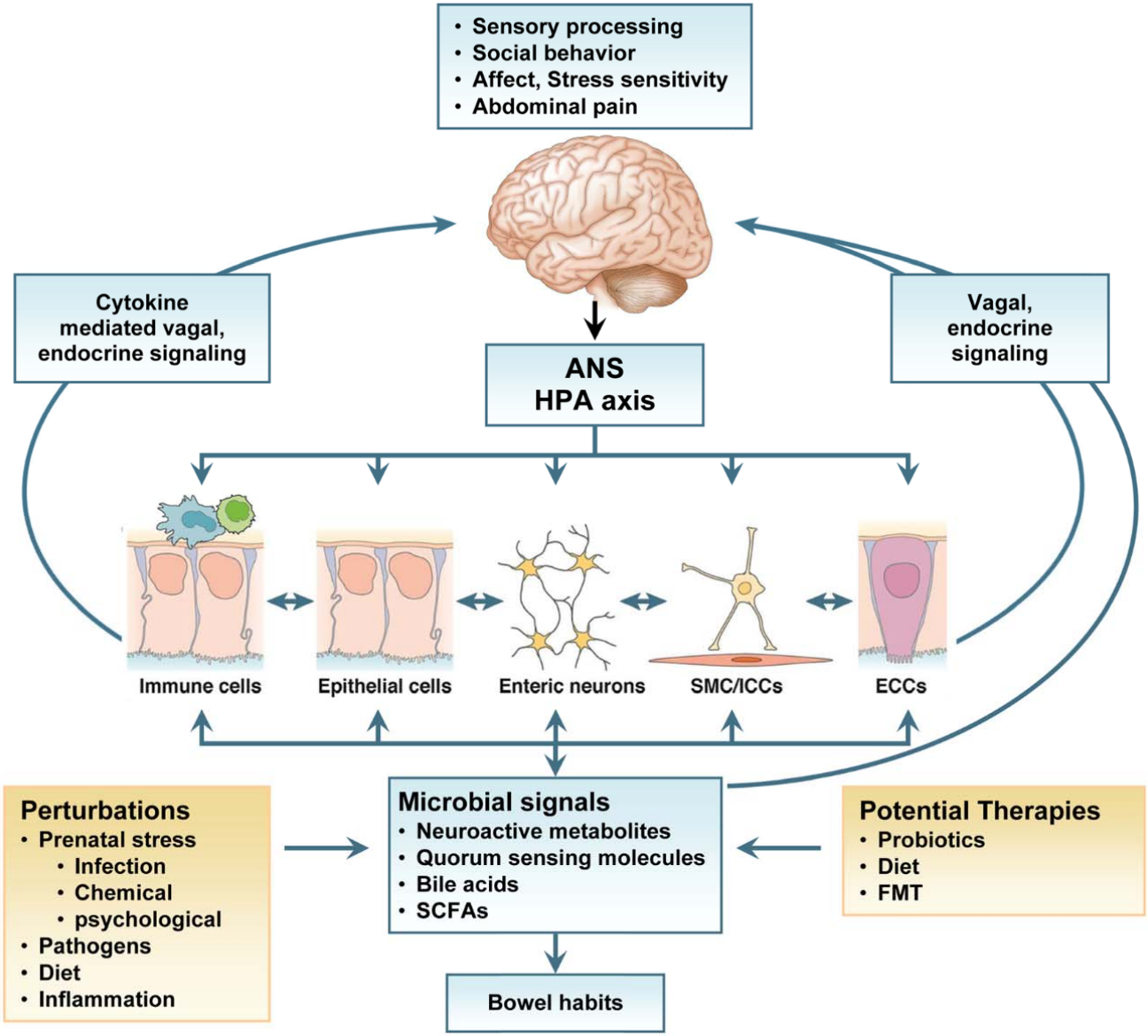
Neurotransmitter	Bacterial Strain	Reference
Serotonin	*Lactococcus lactis* subsp. *cremoris* (MG 1363)	201
	L. lactis subsp. *lactis* (IL1403)	201
	Lactobacillus plantarum (FI8595)	201
	Streptococcus thermophilus (NCFB2392)	201
	Escherichia coli K-12	156
	Morganella morganii (NCIMB, 10466)	202
	Klebsiella pneumoniae (NCIMB, 673)	202
	Hafnia alvei (NCIMB, 11999)	202
Dopamine	*Bacillus cereus*	33
	B. mycoides	33
	B. subtilis	33
	Proteus vulgaris	33
	Serratia marcescens	33
	S. aureus	33
	E.coli	33
	E. coli K-12	156
	M. morganii (NCIMB, 10466)	202
	K. pneumoniae (NCIMB, 673)	202
	H. alvei (NCIMB, 11999)	202
Noradrenaline	*B. mycoides*	33
	B. subtilis,	33
	P. vulgaris	33
	S. marcescens	33
	E. coli K-12	156
GABA	*L. brevis DPC6108*	10
	B. adolescentis DPC6044	10
	B. dentium DPC6333	10
	B. dentium NFBC2243	10
	B. infantis UCC35624	10
	L. rhamnosus YS9	71
Acetylcholine	*L. plantarum*	122
Histamine	*L. lactis subsp. cremoris* (MG 1363)	201
	L. lactis subsp. lactis (IL1403)	201
	L. plantarum (FI8595)	201
	S. thermophiles (NCFB2392)	201
	M. morganii (NCIMB, 10466)	202
	K. pneumoniae (NCIMB, 673)	202
	H. alvei (NCIMB, 11999)	202



- Some gut commensals produce or release neurotransmitters.
- Metabolites may pass from the gut lumen to the blood stream and impact sites distant from the gut.

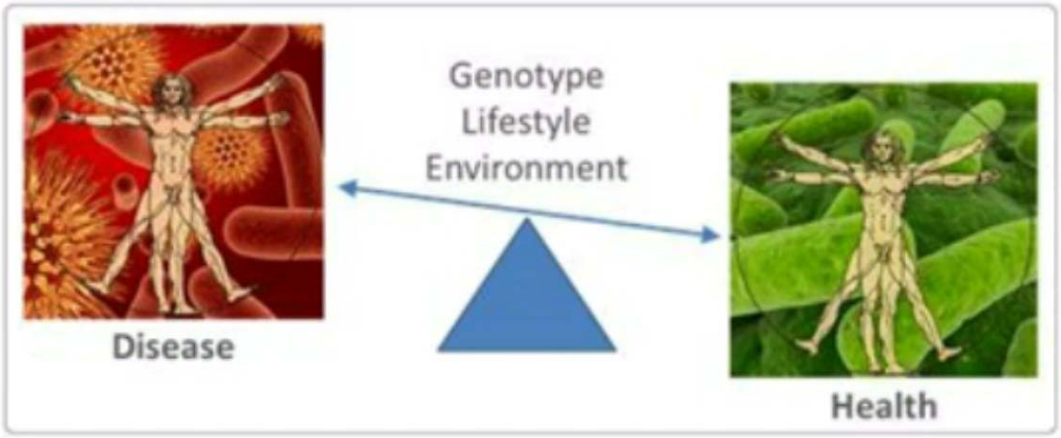
Clarke et al. Mol Endocrin 2014

Gut-Brain axis - autism

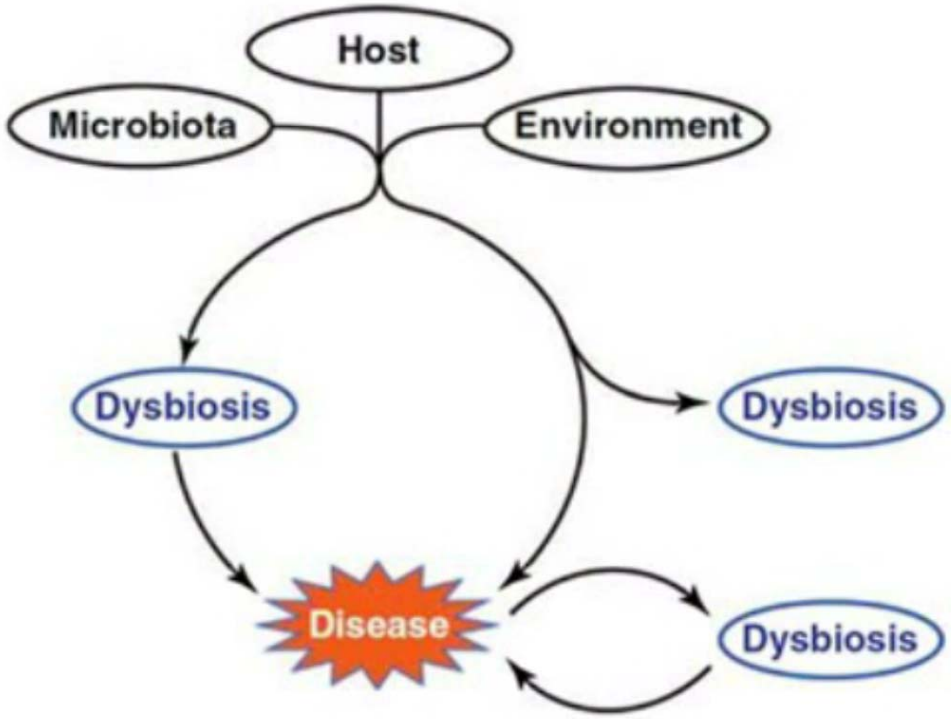


- GI symptoms are a common comorbidity in ASD patients
- Some clinical evidence of dysbiosis in ASD patients
- To be determined whether such changes reflect altered neural control of gut function (motility, secretion) or if changes are primary and affect brain development and function
- Cause versus consequence?

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



Dysbiosis – cause or consequence – and so what?

“Le malade imaginaire”

Molière, 1673

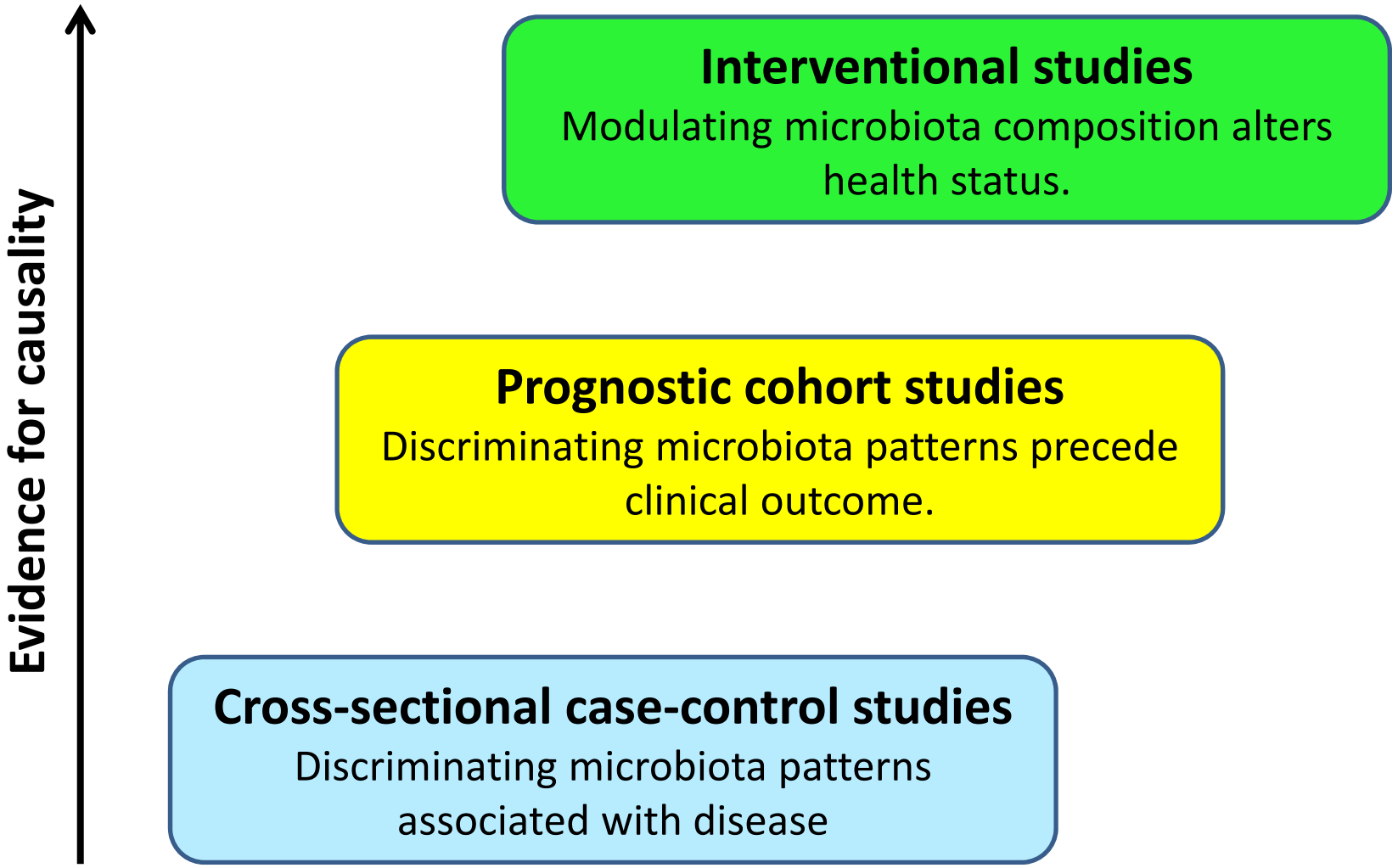
- Prescription of “lavement” for:
  - Digestion, intestinal secretion and bad mood.
- Dates back at least to 100 B.C.

- 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 mcirobiota 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



Take home message

- Gut – Brain – Metabolism should be considered in an integral rather than an individual manner

- Gut microbiota and derived metabolites influence host immunity, nervous system and metabolism.

- Host immunity generates a tolerogenic state between host and gut commensals enabling the host to profit from the vast metabolic potential of gut microbiota.

- Dysregulation of host immunity and dysbiosis may lead to diseases within and beyond the gut.

- Identification of causality between gut dysbiosis and pathology requires:
  - Interventional studies
  - Of note, the bi-directional link between gut, brain and host metabolism makes it likely that an intervention targeting one of them will modify the others at short term. Causality therefore requires long-term resolution of symptoms (**CURE**).