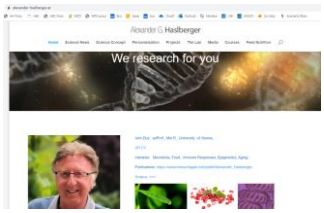
SE Immunologisch und technologisch relevante Aspekte von Lebensmittel 2019

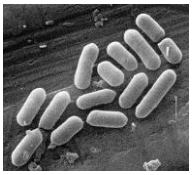


1

IS general
T cell responses
hygiene theory
Allergy intolerance

2

Innate (non-specific) Immunity



- 4 barriers to infection:
  - Anatomic
  - Physiologic
  - Phagocytic
  - Inflammatory

•1st line of defense
•includes chemicals, structure of skin/other epithelia, and mechanisms as well as cells – mainly neutrophils and macrophage

Most MO's are quickly cleared within a few days by innate immunity – **before** adaptive immunities are activated

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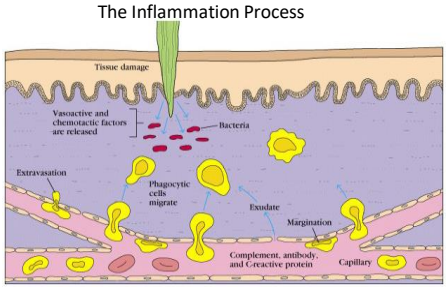
The Inflammation Process



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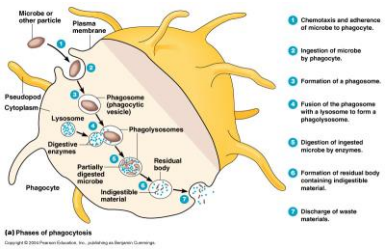

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Adaptive Immunity

Displays four (4) attributes:

1) antibody specificity – distinguishes minute differences in molecular structure to determine non-self antigens.

2) diversity – the immune system can produce a hugely diverse set of recognition molecules which allows us to recognize literally billions of molecular shapes

3) memory – once it has responded to an antigen, the system maintains a memory of that Ag

4) **self-nonself recognition** –the system typically responds only to foreign molecules

***adaptive IR is not independent of innate IR – they're connected**

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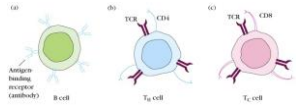
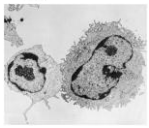
Adaptive Immunity requires 2 major groups of cells:

a. B and T Lymphocytes (B or T cells)



b. Antigen presenting cells (APC's)
-macrophage (MØ)
-dendritic cells (DC)
-B cells



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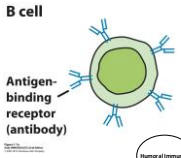
B Lymphocytes:



- Form and mature in bone marrow
- Exhibit antibody receptors on membrane
- Once naïve B cells bind Ag, they divide rapidly to produce:
  - Plasma cells (effector B cells)
  - Memory cells

Humoral Immunity →

Plasma cells are secretory; live only a few days (produce > 2,000 molecules of Ig/sec)

Memory cells have longer life span than naïve B cells

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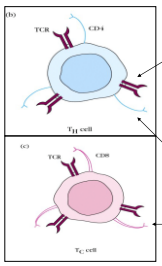
T Lymphocytes



- Formed in bone marrow; migrate to and mature in Thymus gland
- Exhibit unique T-cell Antigen receptors (**TCR**'s) on surface
- **TCR**'s can only recognize Ag with associated with MHC glycoproteins
  - **MHC I** – found on nearly all nucleated cells
  - **MHC II** – found only on APC's

Once T cell binds to Ag, it triggers cell division to form both memory T cells and effector T cells

There are 2 populations of T cells characterized by the type of **CD glycoprotein** found on surface:

T_H – exhibits **CD4**

T_C – exhibits **CD8**

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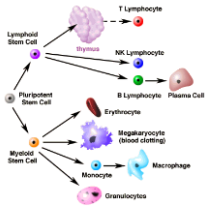
Development



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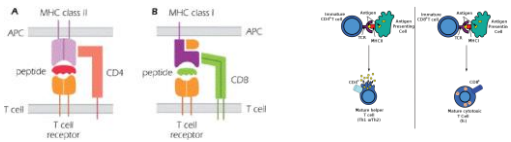
MHC I, MHC II



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The Antigen presentation scenario:

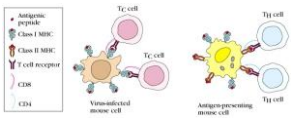

Fig 1-8 Kuby, 4e

Different patterns of cytokines determines types of IR:

-if T_C cell recognizes an Ag/MHC I complex, it divides and differentiates to become CTL

if T_H cell recognizes Ag/MHC II complex, it divides and stimulates B cells, T_C cells, and MØ

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Humoral vs Cell-mediated Immune Response:

Humoral IR: occurs when Ag becomes coated with Ab which brings about the elimination of the foreign body

- -cross-link several Ag's to form clumps -> more easily phago'd
- -bind complement proteins
- -neutralize toxins, viruses, and bacteria from binding target cells

Cell-Mediated IR: occurs when effector T cells are activated

- -activated T_H cells → activate phagocytic cells
  activate B cells to produce Ab
- -activated T_C cells → kill altered self cells (viral infected and tumor cells)

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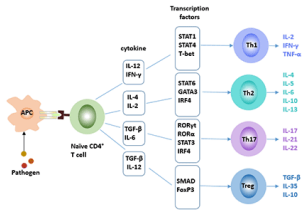
Cytokines and transcription factors drive T cell specification



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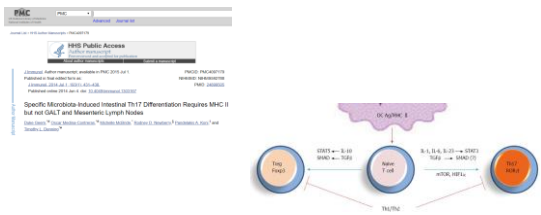

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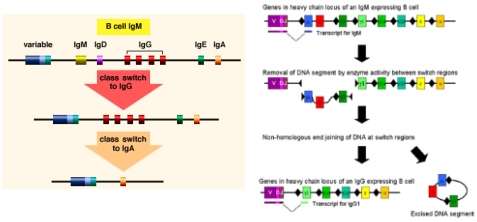
IgM, IgA, IgG,



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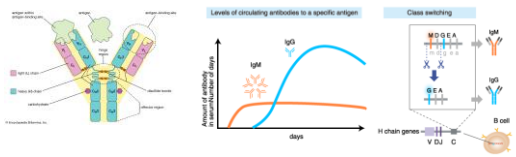

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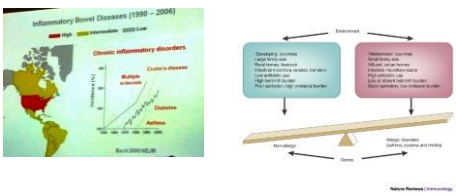
Increases of complex diseases: Do changes of bacterial environment (GI-Microbiota ?) correlate with increase in chronic (inflammatory) diseases ?



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Asthma - Milch vom Bauernhof schützt Kinder vor Asthma und Allergien

Eine Studie an beinahe 15'000 Kindern zeigt, dass das Trinken von Bauernmilch Kinder vor Asthma und Heuschnupfen schützen kann.

Der Konsum von roher Milch birgt jedoch ernste gesundheitliche Risiken und es bedarf weiterer Forschungen für die Entwicklung eines sicheren Lebensmittelprodukts, das einen wirksamen Schutz gegen diese verbreiteten Kinderkrankheiten bietet.

Die Studie, bei der das Institut für Sozial- und Präventivmedizin der Universität Basel federführend war, wurde in der Fachzeitschrift "Clinical and Experimental Allergy" veröffentlicht.

Sämtliche Kinder, welche Milch direkt vom Bauernhof trinken, zeigen denselben Schutzlevel gegen Asthma und Allergien, ungeachtet dessen, ob sie auf einen Bauernhof leben oder nicht"

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Hygiene theory

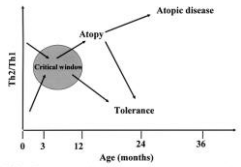

FIG. 1. Development of immune responses in healthy and atopic infants.

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Hygiene theory and microbiota

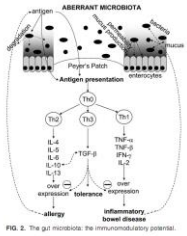

FIG. 2. The gut microbiota: the immunomodulatory potential.

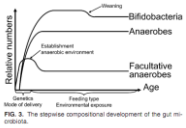

FIG. 3. The stepwise compositional development of the gut microbiota.

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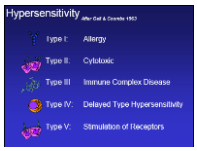
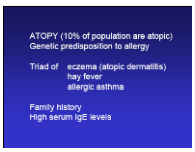
Hypersensitivity, Allergens:
Clemens von Pirquet 1906





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Food Intolerances

The term "food intolerances" otherwise can be used imprecisely. The symptoms may extend from food toxicity to non-specific symptoms such as diarrhea and headache. Triggers can be natural food ingredients like lactose, or synthetic additives such as preservatives.

Enzymatic food intolerance: This intolerance is due to an enzymatic defect in the gastrointestinal tract. The best-known food intolerance is lactose intolerance. It is a β-galactosidase deficiency. Thereby lactose (disaccharide of galactose and glucose) is not metabolized, and reaches the large intestine. There lactose is broken down by bacteria and H_2O, CO_2 and H_2 are produced. This fermentation causes gastrointestinal discomfort.

Pharmacological food intolerance: This incompatibility is caused by vasoactive amines or other substances. A well-known example is the amine histamine. On the one hand histamine is an endogenous substance that also occurs in allergic reactions, and on the other hand histamine occurs mainly in protein-rich foods (e.g., cheese, fish and meat products). Histamine intolerance occurs due to an increased intake of histamine or a defect or malfunction of the histamine-degrading di-Amin oxidase (DAO). In healthy adults, the histamine is released already in the gut of the di-Amin oxidase, whereas in histamine-intolerant individuals, the histamine stays extended in the blood and can lead to non-specific and sometimes very different symptoms. Some common symptoms include headaches, migraines, dizziness, skin itching and redness, arrhythmia, low blood pressure, gastrointestinal complaints, runny nose and frequent sneezing.

Undefined intolerances: In this incompatibility, the mechanisms are not yet identified. These include certain intolerance reactions to food additives, such as "sulphites, nitrites, nitrates, monosodium glutamate and some colorings. Possible symptoms are asthma, rhinitis, urticaria, Itchiness, and migraines."

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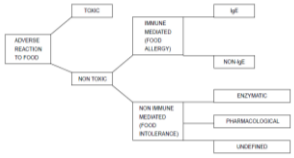
Food intolerances



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Potential allergenicity of protein in food

- If the protein similar to a known allergen, specific IgE may be cross-reactive (recognition of similar epitopes)

  sequence → conformation → cross-reactivity

- How to determine potential allergenicity:

1. stability
2. Compare amino acid sequences by computer programs
3. Recruit potentially at-risk individuals (allergic patients)
4. Perform serum testing, skin prick testing, food challenge.

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Comparison of amino acid sequences

- FASTA and BLAST alignments (used for species homologies) to identify IgE and T cell epitopes?
- Since 1990ies: 8 contiguous amino acid matches
- In 2001: 6 amino acid matches are too short, too many matches; >35% identity over 80 amino acids is useful
- Points of discussion:

1. **Allergen** databases are incomplete, mainly lacking minor allergens
2. **Epitopes** are poorly defined and the relevance of conformational epitopes is not fully established
3. **Analysis of 3D structures:** group proteins into structural families and compare motif recognition patterns

Goodman, R.E. Mol Nutr Food Res, 50: 655-660 (2006).

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Consensus 2005- workshop in Spain

- Short matches are not predictive
- FASTA and BLAST algorithms are efficient
- Structural comparison may be very useful
- There are currently no data to change the **guidelines**

(>35% identity over 80 amino acids)

Risk assessment and food allergy: the probabilistic model applied to allergens Spanjersberg, M.Q.I., Kruizinga, A.G., Rennen, M.A.J., Houben, G.F., Food Chem Toxicol, 45: 49-54 (2007)

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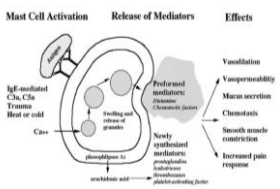
Mast cells



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Tolerance

The oral tolerance is a not- immunological reaction that occurs after the first contact with food antigen. Some animal studies show mechanisms that lead to the absence of the development of oral tolerance. These mechanisms can be transferred to humans and are as follows:

•"Apoptosis of antigen-specific T cells, with consequent loss of their specific immunological function. This mechanism was observed following contact with high doses of antigen.

•Paralysis of the T cells which can occur if the antigen presentation from the epithelial intestinal cells (which function as Antigen presenting cells) is incomplete due to the lack of co-stimulatory molecules.

Defect in the production of the regulatory T cells. The development of these cells is stimulated by external factors such as the intestinal homing of normal bacterial flora after birth. In fact 'germ free' mice are not able to develop normal OT".

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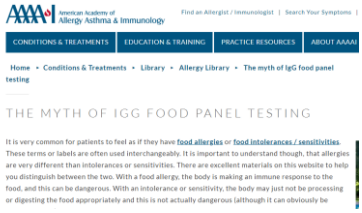
Intolerances ?



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Allergy vs. Intolerance vs. Sensitivity
Food allergies happen when the immune system reacts to a substance, which is usually a protein, in a food or group of foods. Typically, the immune system goes into gear when it detects a harmful substance. It does that by making antibodies. When someone has a food allergy, their immune system identifies a specific protein as harmful and makes antibodies to fight it off. This results in a range of symptoms, including skin rashes and breathing problems.
Food intolerances are not an immune system reaction. They relate to trouble digesting foods. Food intolerances occur due to the lack of an enzyme needed to digest certain foods or, sometimes, as a reaction to additives or naturally occurring compounds in foods. Individuals with food intolerances may be able to eat small amounts of bothersome foods. But, when they have too much, their body reacts. For example, many people with a lactose intolerance find they can drink a small amount of milk with meals or eat yogurt or other foods that are lower in lactose without experiencing any symptoms.
Food sensitivity has no standard medical definition. It can be used to mean anything. Sometimes, this term is used instead of food intolerance, such as a sulfite sensitivity and histamine sensitivity. Other times, it is used as a catch phrase that includes both food allergies and intolerances.

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Helper t cells

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CD4 CD 8

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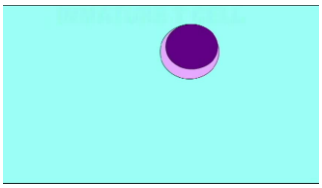
Hygiene theory



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IS of Gastrointestinal tract

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- Structure cells and function of GI tract

Location	Structural Cells	Functions
Esophagus	Squamous epithelial cells	Motility Barrier
Cardia	Columnar epithelial cells Mucous cells Chief cells	Barrier Acid protection
Corpus (body, fundus)	Columnar epithelial cells D cells Chief cells	Mechanical food breakdown Acid and pepsin secretion
Antrum	Columnar epithelial cells Goblet cells Enterochromaffin cells Parietal (oxyntic) cells Chief cells D cells Mucous folveolar (pit) cells Chief cells	Complete digestion Chyme reservoir and release Mucus secretion Acid secretion Histamine release Prostaglandin E_2 secretion Pepsinogen secretion
Small Intestine	Absorptive columnar cells D cells Chief cells M cells Intraepithelial lymphocytes Goblet cells Crypt stem cells Paneth cells	Digestion Absorption Antigen processing and presentation
Colon	Columnar epithelial cells Crypt cells Goblet cells Endocrine cells Stem cells	Water absorption

Middleton 8th edition; gastrointestinal mucosal immunology

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Microfold cells (or M cells) are found in the gut-associated lymphoid tissue (GALT) of the Peyer's patches in the small intestine, and in the mucosa-associated lymphoid tissue (MALT) of other parts of the gastrointestinal tract. These cells are known to **initiate mucosal immunity responses** on the apical membrane of the M cells and allow for transport of microbes and particles across the epithelial cell layer from the gut lumen to the lamina propria where interactions with immune cells can take place.

Paneth cells are a principal cell type of the small intestine epithelium, along with goblet cells, enterocytes, and enteroendocrine cells. When exposed to bacteria or bacterial antigens, **Paneth cells secrete some of these compounds into the lumen of the intestinal gland, thereby contributing to maintenance of the gastrointestinal barrier.**

Undifferentiated intestinal stem cells (ISCs). Recent studies have suggested that ISCs are located either at the crypt base interspersed between the Paneth cells or within the intestinal crypt

Goblet cells are simple columnar epithelial cells that secrete gel-forming mucins,

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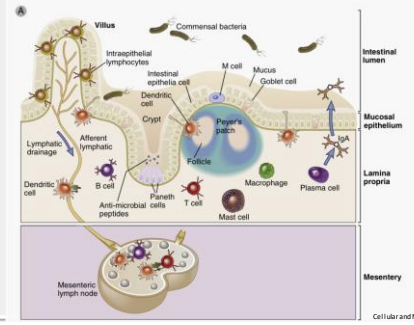

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Mucosal immune system

can be further divided into

1. **The effector arm**
   - phagocytes that engulf and kill microbes; cytotoxic T cells, B cells, helper T cells

2. **The inductive arm**
   - are organized lymphoid structures that bring together naive T cells, B cells, and antigen-presenting cells
     - draining lymph nodes
   - specialized lymphoid structures:
     - PayersPs in small intestine
     - structurally similar lymphoid tissues in the rectum
     - smaller ILFs
     - cryptopatches (precursor to intestinal lymphoid follicles) scattered throughout intestine

Middleton 8th edition; gastrointestinal mucosal immunology

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Innate immunity in gastrointestinal tract

- Intestinal epithelial cells (IEC)
- Antimicrobial peptides
- To l l -like and NOD-like receptors
- Intestinal microbiome
- Innate immune cells

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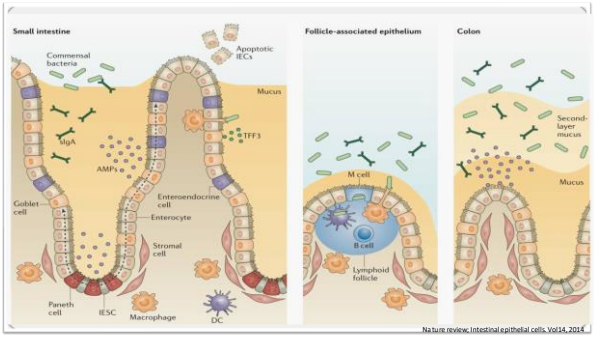

Nature review. Intestinal epithelial cells. Vol14, 2014

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IEC regulation of barrier function

- largest of the body's mucosal surfaces, covering ~400 m2 of surface area with a single layer of cells organized into crypts and villi
- This surface is continually renewed by pluripotent intestinal epithelial stem cells that reside in the base of crypts
- The proliferation, differentiation and functional potential of epithelial cell progenitors is regulated by the local stem cell niche

Nature review. Intestinal epithelial cells. Vol14, 2014

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Extracellular components of IEC barrier

Mucin

- A family of heavily glycosylated proteins that are secreted as large aggregates by mucous epithelial cells
- create a barrier that prevents large particles, including most bacteria

Unstirred layer

- A thin layer of fluid at epithelial cell surfaces that is separated from the mixing forces created by luminal flow and, in the intestine, peristalsis
  - protected from convective mixing forces, the diffusion of ions and small solutes is slowed
  - slows nutrient absorption by reducing the rate

Nature review Immunol. Intestinal mucosal barrier function in health and disease, 2009

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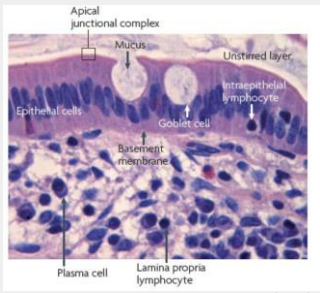

Nature review Immunol. Intestinal mucosal barrier function in health and disease, 2009

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Cellular components of IEC barrier

- In the presence of an intact epithelial cell layer, the paracellular pathway between cells must be sealed.
- This function is mediated by the apical junctional complex, which is composed of the tight junction and subjacent adherens junction
  - supported by supported by a dense perijunctional ring of actin and myosin

Nature reviews Immunol. Intestinal mucosal barrier function in health and disease, 2009

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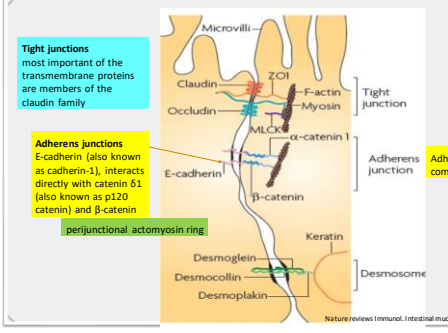

Nature reviews Immunol. Intestinal mucosal barrier function in health and disease, 2009

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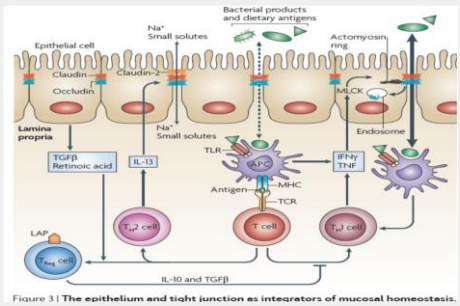

Figure 3 | **The epithelium and tight junction as integrators of mucosal homeostasis.**

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Antimicrobial peptides

- First protective barrier in mucous layer of esophagus: mucin-2 and glycoprotein
- TFF (Trefoil factors) produced by goblet cells: protease-resistant peptides +promote cell survival and migration
- Mucous layer of small intestine: sIgA and antimicrobial peptides

- IL-1,IL-4,IL-6,IL-9,IL-13, TNF, type 1 IFN, neutrophil products, microbial adhesive proteins→increase mucin gene expression

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Defensin families

- Defensins: peptides exert lethal toxic effects on microbes by causing loss of integrity of their outer phospholipid membrane

1. α-defensin: Human neutrophil peptides 1-6, human α-defensin (HD), cryptidins
- HD 5-6 major defensins in small bowel

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Defensin families

2. β-defensin: Human β –defensins 1-4
- HBD 1 multiple location
- HBD 2 upregulated during inflammatory state
- HBD 3 esophagus, oral cavity
- HBD 4 gastric antrum

3. Cathelicidins: humen IL-37 →inc response to bacterial CpG motifs

Cryptidin 4 –against E coli, high level in colon, α-defensin- against Salmonella
Defective- Crohn's disease

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To l l -like and NOD-like receptors

- Pattern recognition receptors (PRRs)
- PRRs recognize pathogen-associated molecular patterns (PA M P s) such as
  - lipopolysaccharide
  - flagellin
  - bacterial DNA and RNA

- PRRs fall into three families
  - To l l -like receptors (TLRs)
  - NOD-like receptors (NLRs)
  - retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs)

Nature reviews Immuno; Role of the gut microbiota in immunity and inflammatory disease. Vol13. 2013

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Toll-like receptors

- Role of TLR in GI

1. Sensing bacteria in intestinal epithelium
2. Sensing intestinal injury
3. Regulate barrier function

Nature reviews Immunol; To l l -like receptor signalling in the intestinal epithelium. Vol 10. 2010

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1. Sensing bacteria by the intestinal epithelium

- Disruption of the epithelial barrier (during ulceration or infection) -> PAMPs to access the surface

A. Expression of TLR in intestine
- TLR2 TL4 low levels by IECs in normal tissue
- TLR3 abundantly in small intestine and colon
- TLR5 predominantly at colon

B. TLR expression by cell lineage in the intestine.

Nature reviews Immunol; To l l -like receptor signalling in the intestinal epithelium. Vol 10. 2010

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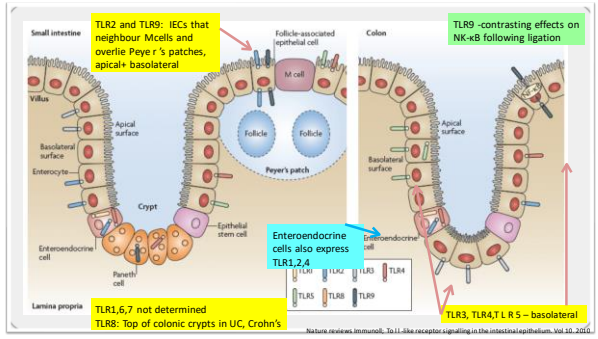

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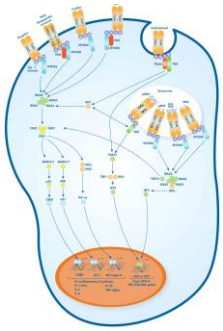
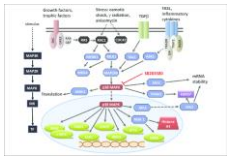


IRAK2 encodes the interleukin-1 receptor-associated kinase 2, one of two putative serine/threonine kinases that become associated with the interleukin-1 receptor (IL1R) upon stimulation. IRAK2 is reported to participate in the IL1-induced upregulation of NF-kappaB.[6]

transforming growth factor-β-activated kinase 1 (TAK1) is a central regulator of cell death and is activated through a diverse set of intra- and extracellular stimuli.



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C. Spatially restricted TLR expression by polarized IECs

- TLR4 - highly expressed on apical side of colonic IECs from patients with active Crohn's disease
- TLR5 - trigger production of cytokines and chemokines, such as IL-8 and CC-chemokine ligand 20 (CCL20), in response to basolateral flagellin
  - luminal flagellin could activate TLR5 only after injury to the epithelial barrier induced by the chemical dextran sodium sulphate (DSS)
- Other TLR-depend on the polarized cell line
  - basolateral or apical exposure of T84 cells or Caco-2 cells, respectively, to LPS results in activation of nuclear factor-κB and IL-8 secretion

Nature reviews Immunol; To l l -like receptor signalling in the intestinal epithelium. Vol 10. 2010

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expression of TLR4 by IECs

- TLR4 signaling- occur at the plasma membrane following binding of LPS to MD2–TLR4 complexes {co-receptor MD2 (known as LY96)}
- IFNγ and TNF induce the transcription of TLR4 and MD2
- IL-4 and IL-13 decrease the responsiveness of IECs to the TLR4 ligand LPS (TH2-type cytokines decrease the expression)

Nature reviews Immunol; To l l -like receptor signalling in the intestinal epithelium. Vol 10. 2010

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Negative regulators of TLR signalling in IECs

- Toll-interacting protein (ToLLIP): intracellular protein inhibits TLR2 and TLR4 signalling through its effect on IL-1R associated kinases (IRAKs)
  - IBD failed to upregulate Tollip expression
- Single immunoglobulin IL-1R-related molecule (SIGIRR; TIR8): negative regulator of IL-1R, IL-33R, TLR4 and TLR9 signalling
  - when deleted-susceptible to intestinal inflammation

Nature reviews Immunol; To l l -like receptor signalling in the intestinal epithelium. Vol 10. 2010

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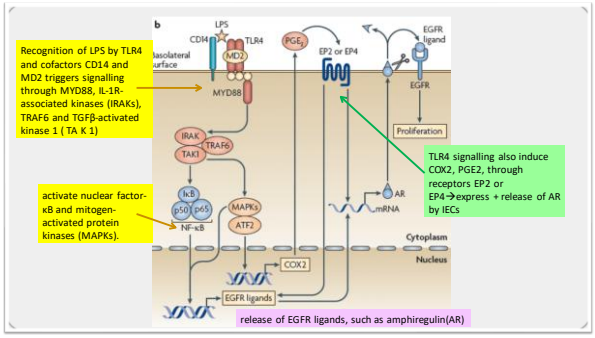

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3. TLRs regulate barrier function

- Activation of **TLR2**→phosphorylation of protein kinase Cα (PKCα) and PKCδ→ reorganization of Zo1 in tight junctions→inc strength of tight junction, inc IEC motility and proliferation
- TLR2 ligand treatment in DSS-induced colitis led to decreased IEC apoptosis and improved tight junction function

Nature reviews Immunol; Toll-like receptor signalling in the intestinal epithelium. Vol 10. 2010

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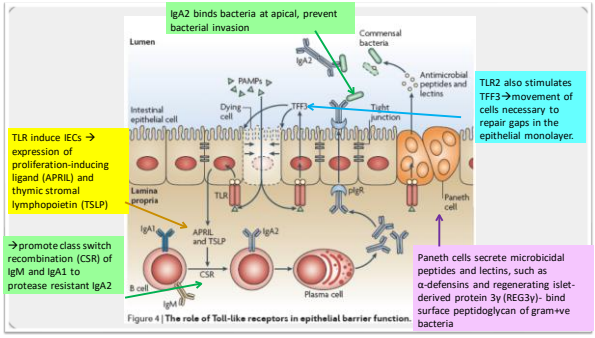

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NOD like receptors (NLRs)

- Nucleotide-binding oligomerization domain-containging protein: NOD1, NOD2
- Intracellular innate immune proteins
  - enable detection of intracellular bacteria
  - promote clearance through initiation of a pro-inflammatory transcriptional programme and other host defence pathways, including autophagy

Nature reviews Immuno; NOD proteins. Vol 14 2014

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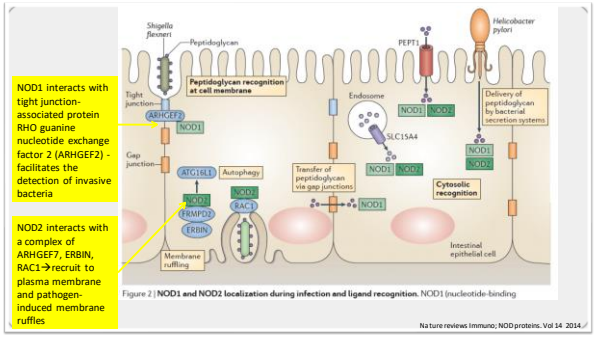

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Consequences of NOD activation

- **Innate immune response**
  - expression of pro-inflammatory immune factors : TNF, IL-6, CC-chemokine ligand 2 (CCL2), neutrophil chemoattractants CXC-chemokine ligand 8 (CXCL8, IL-8) , CXCL2, antimicrobial factors- defensins
- **Adaptive immune responses**
  - combined effect of stromal activation of cytokines, including thymic stromal lymphopoietin (TSLP), and direct sensing of NOD ligands by DCs to drive TH2-type immune responses

Nature reviews Immuno; NOD proteins. Vol 14 2014

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Consequences of NOD activation

- **Antimicrobial functions of NOD proteins**
  - NOD2 -Yersinia pseudotuberculosis, Listeria monocytogenes, Citrobacter rodentium, adherent-invasive E. coli, Staphylococcus aureus
  - NOD1-H. pylori, Legionella pneumophila, Spi1 type 3 secretion system (T3SS) mutant strain of Salmonella spp
- **NOD proteins and autophagy**

Nature reviews Immuno; NOD proteins. Vol 14 2014

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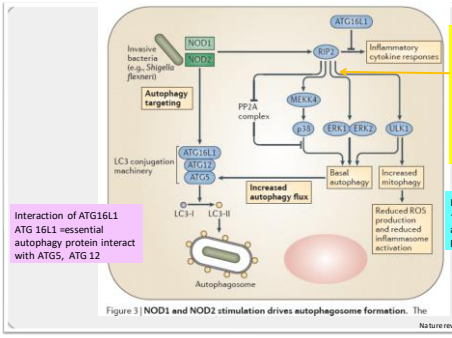

Stimulation of NOD1 and/or NOD2 →RIP2 activation →activating MAPK/ERK kinase kinase 4 (MEKK4) →signalling p38 and/or extracellular signal-regulated kinase 1 (ERK1)+ERK2,→ upregulates basal levels of autophagy

RIP2-dependent ULK1 →restricts inflammasome activation by targeting ROS - producing mitochondria

Interaction of ATG16L1 ATG 16L1 =essential autophagy protein interact with ATG5, ATG 12

Figure 3 | **NOD1 and NOD2 stimulation drives autophagosome formation.** The

Nature reviews Immuno; NOD proteins. Vol 14 2014

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Role of NOD in GI tract

1 Regulation of intestinal barrier function
- fortification of the intestinal epithelial barrier
- promotion of protective and tolerant immune responses within the intestinal mucosa
- regulation of the micobiota
- NOD2
  - maintain integrity of epithelial barrier (potential link to Crohn's disease)
  - regulate expression+secretion of antimicrobial peptides in mouse models
- Mice that are deficient for NOD1 and NOD2 -↓expression of bactericidal lectin regenerating islet-derived protein IIIγ (REGIIIγ) and intact goblet cells

2 Regulation of immune homeostasis in the gut
3 Regulation of the microbiota

Nature reviews Immuno; NOD proteins. Vol 14 2014

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Roles of NOD in GI tract

1 Regulation of intestinal barrier function

2 Regulation of immune homeostasis in the gut
- indirectly regulate T cell populations
- NOD1 and NOD2 -drive a protective early TH17 cell response following bacterial infection
- regulate effector functions of mononuclear phagocyte (induce CCL2 expression from gut stroma→recruits IL-12-producing LY6Chi monocytes)

3 Regulation of the microbiota
- both NOD2-deficient and RIP2-deficient mice →dysbiosis→colitis
- In human studies, no significant differences in microbial community structure from individuals with Crohn's disease-associated NOD2

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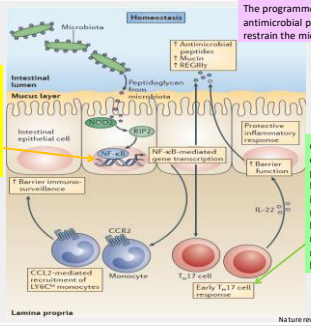
Normal homeostasis



The programme - stimulate production of antimicrobial peptides and mucin→ restrain the microbiota

key role of NOD in intestinal homeostasis =detecting peptidoglycan that is released from gut microbiota and driving a physiological inflammatory programme through RIP2 → NF-κB activation

early T TH17 cell response enhances barrier protection by inducing IL-22 and regenerating islet-derived protein IIIγ (REGIIIγ), CC-chemokine ligand 2 (CCL2)-mediated recruitment of LY6Chi monocytes enhances barrier surveillance.

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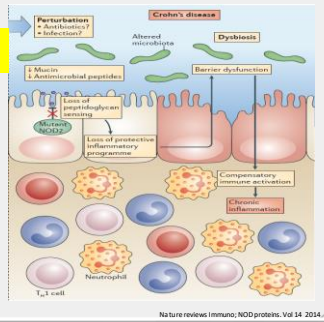
Crohn's disease

some perturbation: antibiotics, infection→alters protective inflammatory programme→a breakdown in intestinal barrier and potentially to dysbiosis



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Intestinal microbiome

- Symbiotic relationship
- 4 phyla predominate in colon: *Firmicutes, Bacteroides, Actinobacteria, Proteobacteria*
- Alteration = **dysbiosis**
  - from local/systemic allergy, autoimmunity
  - associate with distinct pattern
- Mice with lower level of segmented filamentous bacteria- reduced number of Th17 cells
- Mice with *Bacteroides fragillis* or *Clostridium species*-induction of Tre g cells

Middleton 8th edition, gastrointestinal mucosal immunology

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Current understanding of the role of gut microbiota

1. Development of intestinal immune system
2. Protection from infection
3. Protection or induction of IBD
4. Extra-intestinal disease

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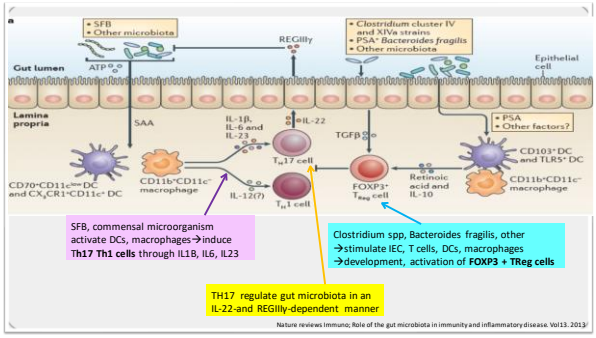

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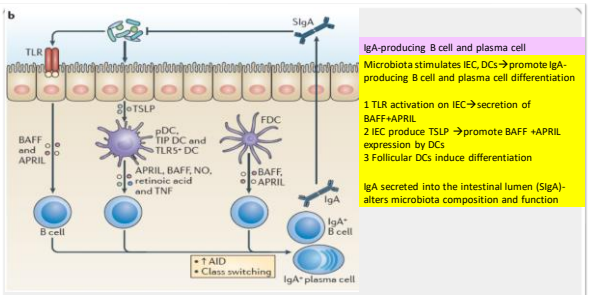

IgA-producing B cell and plasma cell

Microbiota stimulates IEC, DCs→promote IgA-producing B cell and plasma cell differentiation

1 TLR activation on IEC→secretion of BAFF+APRIL
2 IEC produce TSLP →promote BAFF +APRIL expression by DCs
3 Follicular DCs induce differentiation

IgA secreted into the intestinal lumen (SIgA)-alters microbiota composition and function

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Current understanding of the role of gut microbiota

1. Development of intestinal immune system
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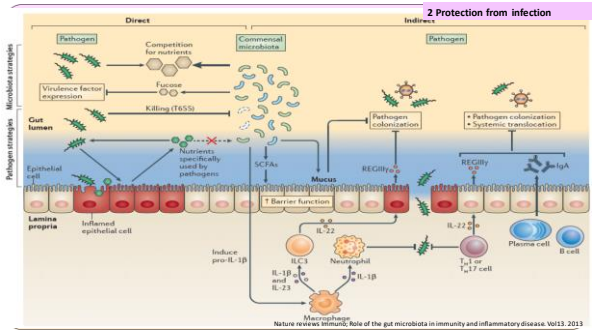
2 Protection from infection



Nature reviews Immuno; Role of the gut microbiota in immunity and inflammatory disease. Vol13. 2013

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3 Protection or induction of IBD

Commensal bacterium	Host	Genotype	Disease	Receptors	Possible mechanism	Refs
Commensal microbiota that protect against IBD						
Clostridium spp. clusters IV and XIVa	Mouse	Wild-type	DSS colitis	Unknown	Induction of T_{reg} cells	19
Altered Schaedler flora	Mouse	Wild-type	DSS colitis	MYD88 and TRIF	Induction of T_{reg} cells	20
Bacteroides fragilis	Mouse	Wild-type	TNBS colitis	TLR2, MYD88	Induction of T_{reg} cells via polysaccharide A	21
Bacteroides vulgatus	Mouse	*Il2*$^{-/-}$	Spontaneous colitis	Unknown	Suppression of *Escherichia coli*-triggered colitis in *Il2*$^{-/-}$ mice	120
Faecalibacterium prausnitzii	Human	NA	IBD	Unknown	Induction of IL-10 by PBMCs	148

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Commensal microbiota that promote the development of IBD						
E. coli	Mouse	*Il10*$^{-/-}$	Spontaneous colitis	Unknown	Monocolonization of germ-free mice induces colitis	119
E. coli	Mouse	*Il2*$^{-/-}$	Spontaneous colitis	Unknown	Monocolonization of germ-free mice induces colitis	96, 120
Enterococcus faecalis	Mouse	*Il10*$^{-/-}$	Spontaneous colitis	Unknown	Monocolonization of germ-free mice induces colitis	119
B. vulgatus	Rat	*HLA-B27–B2m* transgenic	Spontaneous colitis	Unknown	Monocolonization of germ-free rats induces colitis	96
B. vulgatus	Mouse	*Il10r2*$^{-/-}$*Tgfbr2*$^{-/-}$	Spontaneous colitis	Unknown	Colonization of antibiotic-treated mice triggers colitis	122
Bacteroides thetaiotaomicron	Mouse	*Il10r2*$^{-/-}$*Tgfbr2*$^{-/-}$	Spontaneous colitis	Unknown	Colonization of antibiotic-treated mice triggers colitis	122
B. thetaiotaomicron	Rat	*HLA-B27–B2m* transgenic	Spontaneous colitis	Unknown	Monocolonization of germ-free rats induces colitis	95
Bacteroides unifirmatis	Mouse	*Il10r2*$^{-/-}$ *Tgfbr2*$^{-/-}$	Spontaneous colitis	Unknown	Colonization of antibiotic-treated mice triggers colitis	122
*Klebsiella pneumoniae***	Mouse	*Tbx21*$^{-/-}$*Rag2*$^{-/-}$	Spontaneous colitis	Unknown	Other commensal bacteria are required for the induction of colitis	123,124
*Proteus mirabilis***	Mouse	*Tbx21*$^{-/-}$*Rag2*$^{-/-}$	Spontaneous colitis	Unknown	Other commensal bacteria are required for the induction of colitis	123,124
*Helicobacter typhlonius**	Mouse	*Tbx21*$^{-/-}$*Rag2*$^{-/-}$	Spontaneous colitis	Unknown	Transmissible to non-colitogenic TRUC mice	125
*Prevotellaceae***	Mouse	*Nlrp6*$^{-/-}$, *Asc*$^{-/-}$ or *Casp1*$^{-/-}$	DSS colitis	Unknown	Impaired IL-18 signalling promotes pathobiont expansion	126
TM7**	Mouse	*Nlrp6*$^{-/-}$, *Asc*$^{-/-}$ or *Casp1*$^{-/-}$	DSS colitis	Unknown	Impaired IL-18 signalling promotes pathobiont expansion	126
Bilophila wadsworthia	Mouse	*Il10*$^{-/-}$	Spontaneous colitis	Unknown	Consumption of a diet composed of milk-derived fat induces pathobiont expansion	128

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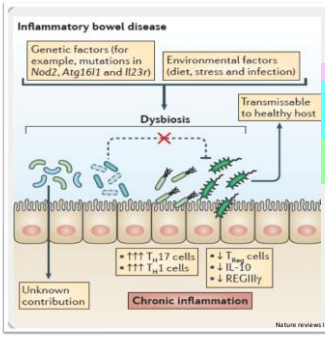

IBD

combination of genetic factors (eg. Nod2, Atg16l1, iL23r) and environmental factors (eg. infection, stress, diet) result in disruption of the microbial community structure→ *"dysbiosis"*

Dysbiosis→accumulation of colitogenic pathobionts→ chronic inflammation involving hyperactivation of TH1, TH17 cells.

Nature reviews Immuno; Role of the gut microbiota in immunity and inflammatory disease. Vol13. 2013

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Immune modulation by gut microbiota

Immune modulation by metabolites

1. Short-chain fatty acids (SCFA)
2. Aryl hydrocarbon receptor (AHR) ligands
3. Polyamines

Immune modulation by microbial components

1. Polysaccharide A (PSA)
2. Formyl peptides
3. Heptose-1,7-biphosphate (HBP)

Michelle G, Nature review Immunol. June 2016

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Table 1 | Microbial metabolites or components that are implicated in disease

Human disease and preclinical models	Microbial metabolites or components	Refs
Allergic and immune disorders		
Asthma	SCFAs	40,45
Inflammatory bowel disease	SCFAs	42–44,53
	B vitamins	53
Cancer		
Colorectal cancer	SCFAs	53,118,119
	B vitamins	53
	N^1,N^{12}-diacetylspermine	80
Gynaecological and reproductive disorders		
Bacterial vaginosis and other sexually transmitted infections	Polyamines	120
	HBP	100
Preterm labour	SCFAs	50
Metabolic disorders		
Cardiovascular disease	TMAO	121
Kidney disease	SCFAs	122
	p-Cresol	123
Obesity and metabolic syndrome	TMAO	121
Type 2 diabetes	TMAO	121
Neurological disorders		
Autism spectrum disorder	4-EPS	124
Central nervous system dysfunction	SCFAs	52,125
Other gastrointestinal disorders		
Infectious colitis (*Clostridium difficile*)	Bile acids	126

4-EPS, 4-ethyl phenol sulfate; HBP, D-glycero-β-D-manno-heptose-1,7-biphosphate; SCFAs, short-chain fatty acids; TMAO, trimethylamine N-oxide.

Michelle G, Nature review Immunol. June 2016

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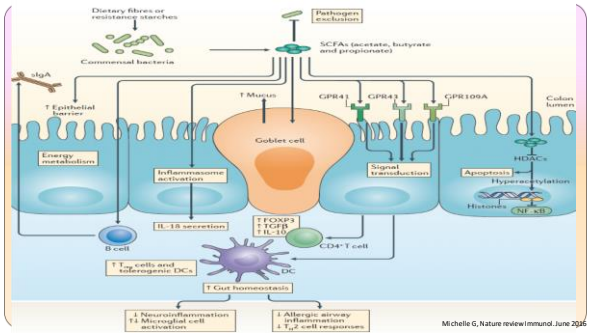

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Innate immune cells

- Eosinophils
- Mast cells
- ILC and multifunctional IgA+ plasma cells
- Macrophages

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Innate lymphoid cells

- Lymphoid tissue inducer (Lti) cells required for LN formation and produce IL-17, IL-22
- Mix NK+LTi characteristic cells- express CD 56, CD 127, produce IL22
- Natural helper , innate halper type2, nuocyte→produce Th2 cytokine IL-5 IL-13
  - Production of IL-5, ILC recruit eosinophils

- IgA –producing B cells produce antimicrobial agents TNF-α, inducible NO syntase→maintain homeostasis of gut microbiota

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Innate lymphoid function

- **Group 1: NK cells, innate lymphoid cell subset 1**
  - Produce TH1 cell-associated cytokines IFNγ and TNF in response to IL-12 and/or IL-15
  - possible role in intestinal inflammation in murine colitis models and human IBD
- **Group 2: ILC2**
  - produce TH2 cell-associated cytokines IL-5 and IL-13, supported by IL-25, IL-33 and TSLP
  - early innate response to intestinal helminth infection, lung promote airway hyperresponsiveness or tissue repair in mouse models
- **Group 3: ILC3**
  - produce TH17 and TH22 cell-associated cytokines (IL-17A and IL-22) in response to stimulation by IL-23
  - IL-22 protect epithelium following injury or infection by bacterium
  - IL-17 pro-inflammatory effect, implicated in mouse colitis and human IBD

Nature review: intestinal epithelial cells. Vol14, 2014

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Macrophage

- GI –largest reservoir of mononuclear phagocytes
- Resident macrophages –highly phagocytic, generating inflammatory response without damage surrounding tissue

- Innate signaling molecule such as MyD88 and TRIF adapter proteins **decreases** in macrophage →explain TLR non-responsiveness despite activation

- Despite of anergic phenotype, macrophage participate in host defense (regulate inflammatory response, scavenge debris, microbial killing)

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Dendritic cells

- Lamina propria contain a dense network of DC

- A subset of DC expressing CD11c and CD103 markers→express high levels of flagellin sensor TLR5, but low TLR4
  - TLR5 necessary for detection of Salmonella typhimurium
- Activation of TLR5→lead to production of IL-23 and ILC expression of IL-17 and IL-22 (antimicrobial defense cytokine)

- Also responsive to TLR3 TLR7 TLR9 stimulation- important for antiviral immunity

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Adaptive immunity

- Functional anatomy of adaptive immunity in GI tract
- Humoral immunity
- Cell-mediated immunity

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Functional anatomy of adaptive immunity

- Initiated in collections of lymphocytes and APC closely associated with the mucosal epithelial lining of bowel and in mesenteric LN
- Gut-associated lymphoid tissues adjacent to mucosal epithelium referred to as GALT
  - most prominent G A LT are Peyer's patches, found mainly in distal ileum or isolated follicles in appendix and colon
- GALT-different from LN
  - ratio of B:T cells 5 times higher
  - not encapsulated
  - independent routes of Ag delivery

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Lymphoid site of immune response

- G A LT
- Mesenteric LN –serve some of the same function as GALT
  - correct lymph-borne Ag
  - 100-150 LN between membranous layer of mesentery
- Lingual and palatine tonsils-sites of immune response in oral cavity

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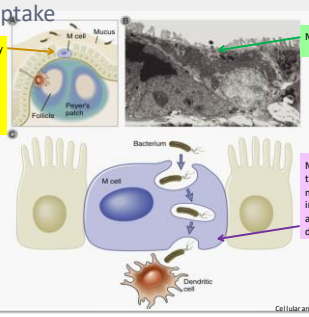
Antigen uptake

major pathway of antigen delivery from lumen to GALT is through specialized cells called microfold (M) cells
- found in small bowel epithelium overlying Peye r 's patchs and lamina propria lymphoid follicles



M cells have shorter villi

M cells engage in transport of microbes or molecules across barrier into GALT, where they are handed off to dendritic cells

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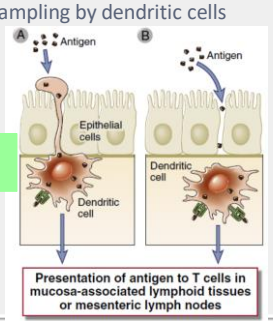
Antigen sampling by dendritic cells

Some DC extend dendritic processes between IEC into lumen to sample antigens



Other present in lamina propria, sample antigens that derived from lumina contents through the epithelial barrier

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Epithelial cells

- Epithelial cells of the GI tract are also capable of presenting Ag to T cells
- During inflammatory states, epithelial cells of the esophagus and small intestine upregulate MHC II and can activate CD4+ T cell

Middleton 8th edition; gastrointestinal mucosal immunology

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Antigen presentation

- APCs in the GI tract include
  - professional APCs such as **DCs, B cells, macrophages**
  - nonprofessional APCs such as **epithelial cells**
- Different DC phenotypes are specialized to respond to specific inflammatory stimuli
  - surface markers: **CD11b, CD8α, and CCR6**

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Humoral immunity

- Major function is to neutralize luminal microbes
- mediated mainly by IgA produced in the GALT
- IgA is produced in larger amounts than any other antibody isotype
  - because of large number of IgA-producing plasma cells in GALT, (80% of all Ab-producing plasma cells in the body)
    - selective induction of IgA isotype switching in B cells in GALT and MLN
    - selective gut-homing properties of IgA-producing plasma cells

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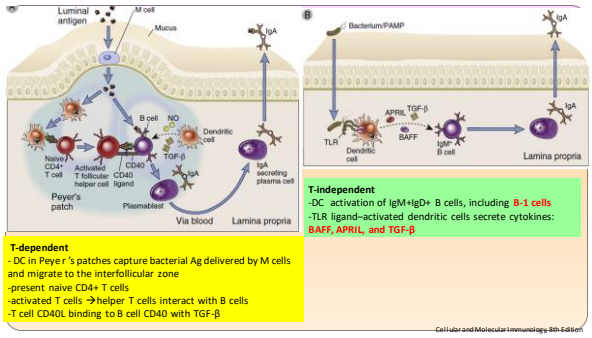

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Cell-mediated immunity

- T cells scattered throughout lamina propria and submucosa and within Peyer 's patches
- Different subsets of effector T cells
  - Th17 cell
  - Th 2 cells

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T-cell-mediated immunity

Th17 cells
- Rich in lamina propria of the small bowel, whereas the colon is not
- depends on colonization with a certain phylum of bacteria (segmented filamentous bacteria) in the postnatal period
- Required for protection of *Citrobacter rodentium*
- 2 signature cytokine: IL-17, IL-22-induce mucin and B-defensins which protect epithelial cells

Th2 cells
- Intestinal helminthic infections induce strong TH2 responses
- cytokines IL-4 and IL-13

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Regulation of GI mucosal immunity

- Regulatory T cells abundant in GALT
  - prevent inflammatory reactions against commensal microbes
- proportion of **FoxP3+ Treg**: 2-fold greater in lamina propria than other peripheral lymphoid tissues
- Factors generate Tre g : CD103+ DC, local production of retinoic acid, TGF-β
- Cytokines: TGF-β, IL-10, IL-2→play role in maintaining homeostasis
- selective deletion of the *Il10* gene in FoxP3+ cells leads to severe colitis

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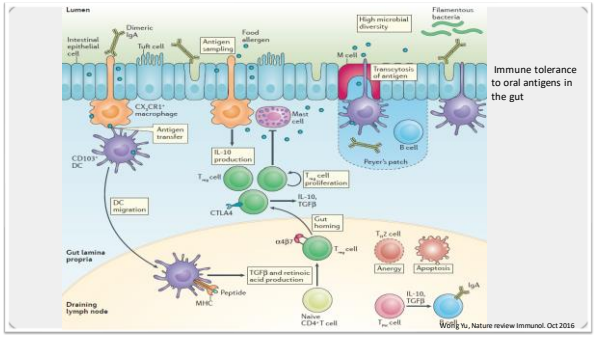

Immune tolerance to oral antigens in the gut

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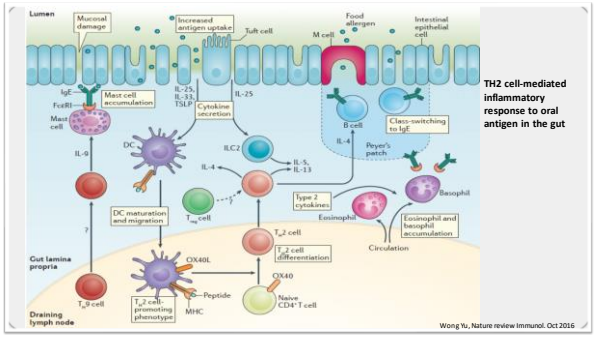
Mucosal immunity related to diseases

Food allergy
Inflammatory bowel disease
Celiac disease

120

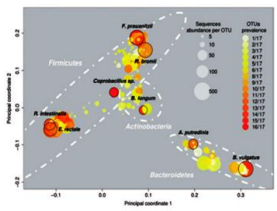


TH2 cell-mediated inflammatory response to oral antigen in the gut

121

126

Microbiota: the role of the distribution of groups (and their functions ?)

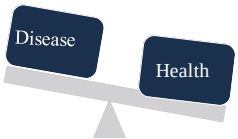


127

Microbiome

Microbiome *- a collection of microbial genomes*
Microbiota *– a collection of microbes*

- As many bacteria as host cells in human body
- 150x more bacterial genes than our human genome

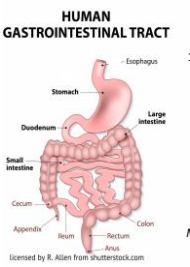


128

GI Microbiota

Stomach & Duodenum
10^1– 10^3 CFU/mL
Helicobacter
Streptococcus

Jejunum & Ileum
10^4 – 10^8CFU/mL
Bacteroides
Streptococcus
Lactobacillius
Bifidobacteria
Fusobacteria



Colon
10^{10}– 10^{12} CFU/mL
Bacteroides
Prevotella
Facaelbacterium
Ruminococcus
Roseburia
Clostridium
Bifidobacteria
Collinsella
Desulfovibrio
Bilophila
Akkermansia
Methanobrevibacter

7

129

Microbiota Functions

- Protective functions
- Structural functions
- Metabolic functions
  - Fermenting dietary fiber into short-chain fatty acids
  - Synthesizing vitamins

Grenham S, Front Physiol, 2011

8

130

"Core" Microbiota

- *Bacteroidetes* (22,9 %)
- *Firmicutes* (64 %)
  (32 % of *C. Cluster IV*, 36 % of *C. Cluster XIVa* and 5 % of *Lactobacilli)*

  (Mariat et al., 2009)
- *Actinobacteria* (1- 4 %)
- Verrumicrobiales (1- 4 %)
- Archaeal domain (1- 2,5 %)
- Eukaryotic microorganisms (< 0,1 %)

  (Gerritsen et al., 2011)

131

Variation in microbiota structure is high

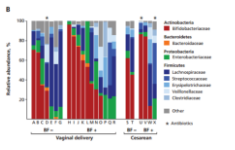
Despite high variation, GI microbiota depend on :

1. Individuum
2. Area and lifestyle
3. Diet
4. Interventions

132

Ways of delivery and microbiota: a long lasting difference

Infants born by elective cesarean delivery had particularly low bacterial richness and diversity. formula-fed infants had increased richness of species, with overrepresentation of *Clostridium difficile*.



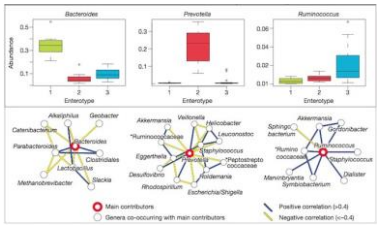
CHILD involves more than 10 000 people, including 3 500 infants

133

We are not born sterile !

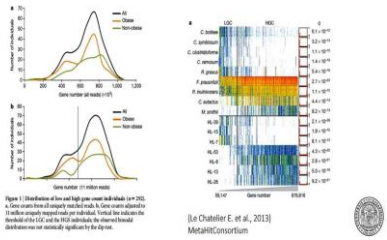
134

Enterotypes ?



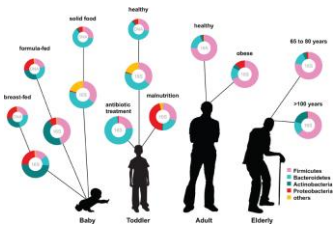
135

GI microbiota: Diversity of groups and functions important for health



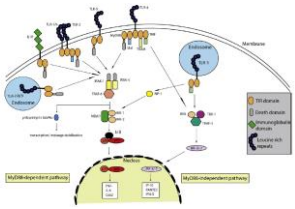
136

Aging and Microbiota



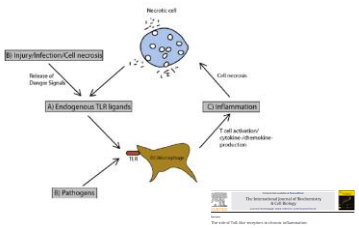
137

Cooperation between microbiota and the I.S.: TLRs, adaptor molecule, MyD88,



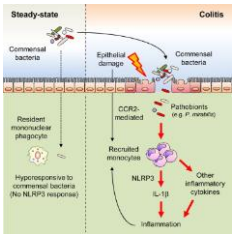
138

Inflammation: TLR ligands from necrotic cells and high fat diet (via LPS) may activate TLRs



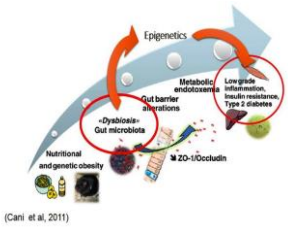
139

Damage of gut wall: Microbiota induce NRLP3 inflammasome and inflammation



140

Bacterial cell wall components and Inflammation: dysbiosis, LPS and gut permeability; obesity as a model

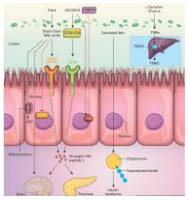


(Cani et al, 2011)

141

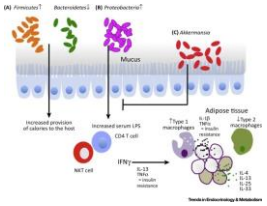
Endotoxins, saturated fats/ chylomicrons trigger inflammation, insulin resistance;
SCFAs may trigger GLP1 activation

GLP1: incretin improves DMII and obesity



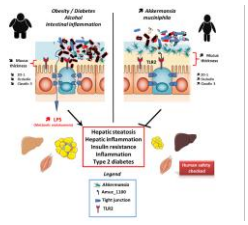
142

Obesity: Firmicutes: Bacteroidetes; Akkermansia and the cell wall



143

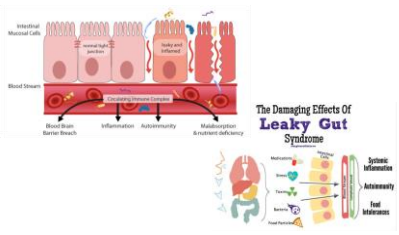
Obesity and Alcohol induce intestinal inflammation, Akkermansia muciniphila/ TLR2 increase mucus, improve tight junction



P Cani, W de Voss; 2017

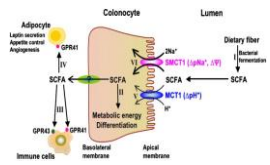
144

leaky gut: a major health problem



145

Microbiota metabolites: SCFAs bind to G-Protein-Receptors GPR 41/43 (FFARs)



Anti-inflammatory;
Inhibition of NFκB

(Huster et al., 2013; Flint et al., 2009, Nature Rev)

146

Microbiota and fermentation products e.g. SCFAs

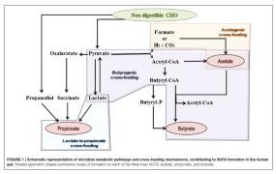
Clostridial cluster IV (Rumminococcaceae)	*Clostridial cluster XIVa* (Lachnospiraceae)
Faecalibacterium prausnitzii *Butyricoccus* *Clostridium Leptum*	*Eubacterium hallii* *Anaerostipes coli* *Roseburia spp.* *E. rectale spp.*
Resistent starch	*Non starch Polysaccharides*

(Louis and Flint, 2009, FEMS)

147

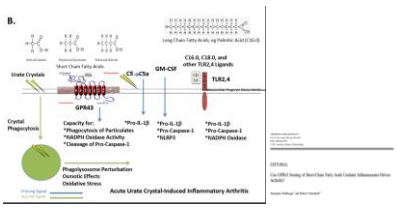
147

Pathways and cross feeding for SCFAs/ Butyrate



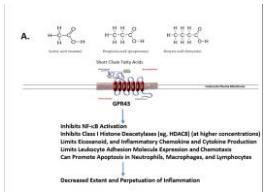
148

TLR2, TLR4 ligands (endotoxins, long chain fatty acid) trigger inflammation, GPR43 interfers?



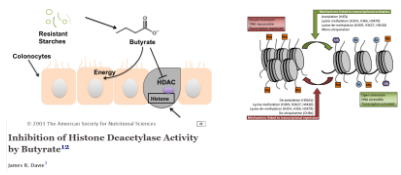
149

SCFAs via GPR43 (GPR41) inhibit NFkB and inflammation



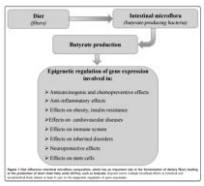
150

Butyrate and epigenetic histone modulation



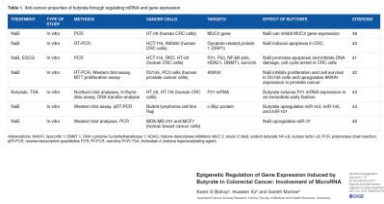
151

Butyrate and epigenetics



152

Butyrate: apoptosis, autophagy, mi- RNAs regulating inflammation, vitro



Epigenetic Regulation of Gene Expression Induced by Butyrate in Colorectal Cancer: Involvement of MicroRNA

153

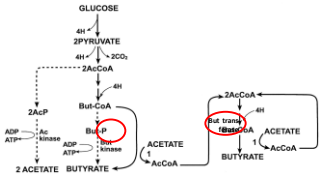
Butyrate: where and how much ?

For good reason it is not possible with current technologies to perform direct measurements of the variation in the butyrate concentration in the portal vein of human subjects, but short-chain fatty acid levels in portal blood from sudden-death victims, subjects undergoing emergency surgery or planned surgery have indicated a higher gut production and absolute and relative concentration of butyrate in non-fasted as compared with fasted human subjects

The gut microbiota influences blood-brain barrier permeability in mice

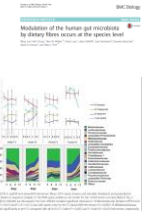
154

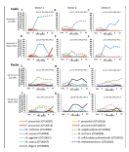
Marker for Bacterial Produced Butyrate



155

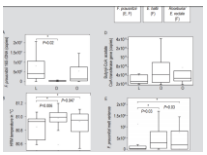
Microbiota and SCFA responses vary very individually





156

SCFAs producers, phylotypes differ in obese, diabetes

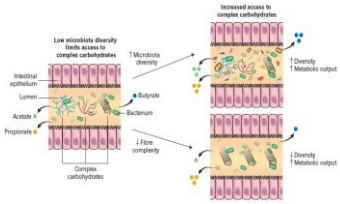


157

SCFAs in elderly: Decrease in SCFAs producers and „Butyrate Gen producing gene" in elderly

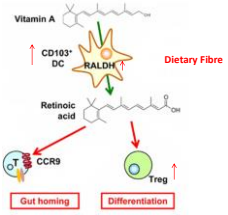
158

Diet dictates the production of SCFAs, diversity of the microbiota, many types of complex carbs



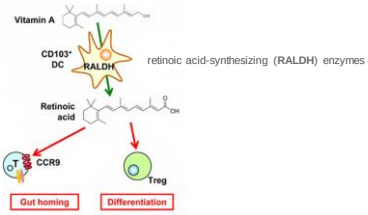
159

Dietary fibre modulate CD103⁺ DCs proportion and activity



160

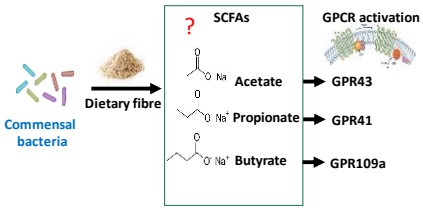
CD103⁺ DCs are key inducer of Treg in the gut



161

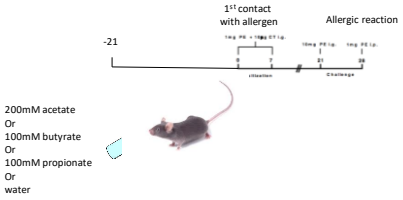
Mechanism of action of fibre: Short-chain fatty acids (SCFAs)?

- SCFAs are major metabolites produced by the microbiota



162

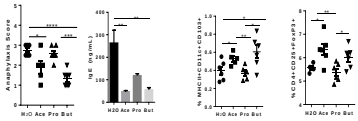
Role of SCFA in food allergy development



163

SCFAs effects in peanut allergy

200mM acetate, 100mM butyrate, 100mM propionate for 3 weeks in drinking water



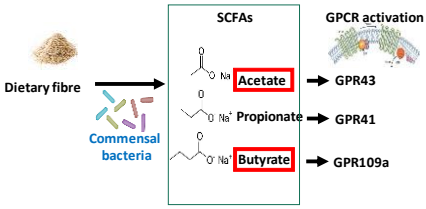
Acetate and Butyrate have beneficial effects:
↓ Anaphylaxis
↓ IgE

Acetate and Butyrate have beneficial effects:
↑ CD103⁺ DC
↑ Treg

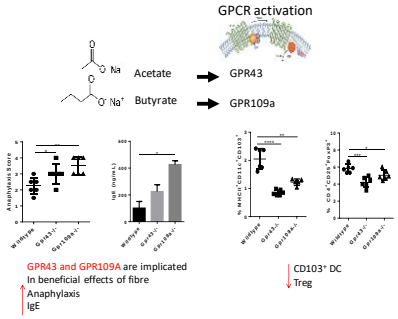
164

Mechanism of action of fibre: Short-chain fatty acids (SCFAs)?

- SCFAs are major metabolites produced by the microbiota

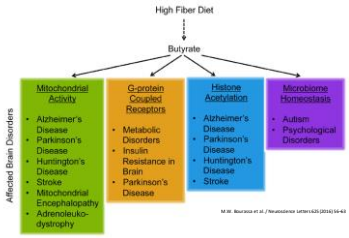


165



166

Butyrate and neuro-epigenetics



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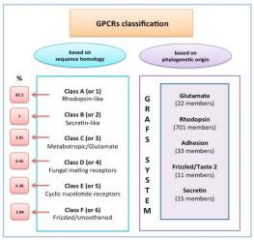
GPRs



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GPRs and therapy, still many unclear

GPR41 AND GPR43 EXPRESSION IS TISSUE-SPECIFIC

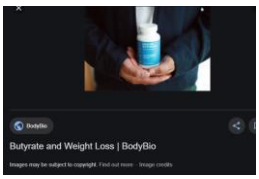
GPR41 AND GPR43 AS POTENTIAL THERAPEUTIC TARGETS FOR OBESITY, COLITIS, ASTHMA, AND ARTHRITIS

REPORTS ON GPR41 AND GPR43 KNOCKOUT MICE PHENOTYPES ARE IN CONSISTENT

Zhiwei Ang and Jeak Ling Ding Front. Imm. 2016*

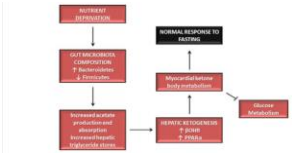
169

Butyrate salts



170

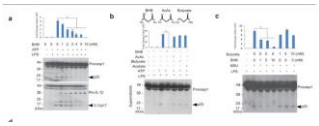
Microbiota regulate not only SCFAs but also Ketone bodies in caloric restriction, BHB



Crawford PA et al, 2009). Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. *Proceedings of the National Academy of*

171

Ketone body β-hydroxybutyrate blocks the NLRP3 inflammasome-mediated inflammatory disease (caspase subunit)

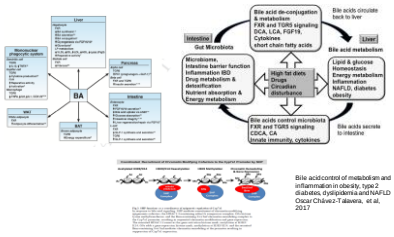


Ketogeneic diet may improve inflammation via epigenetics, but can also lead to an overload of LPS thru high SFA and low vegtable intake

Yun-Hee Youm et al. Nat med. 2015

172

Microbiota modulated Bile acids are epigenetically active and via FXR regulate inflammation

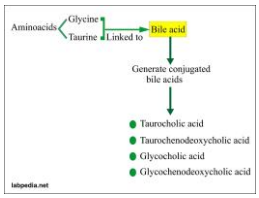


Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia and NAFLD Oscar Chávez-7 aiaxera, et al. 2017

173

Bile acids

Primary bile acids are synthesized by the liver. Secondary bile acids result from bacterial actions in the colon. In humans, taurocholic acid and glycocholic acid (derivatives of cholic acid) and taurochenodeoxycholic acid and glycochenodeoxycholic acid (derivatives of chenodeoxycholic acid) are the major bile salts in bile and are roughly equal in concentration



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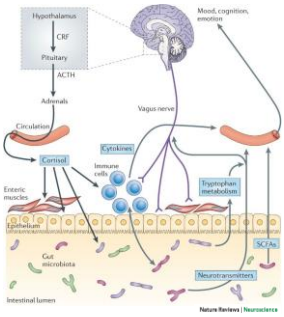
Gut-Microbiota-Brain Communication



Cryan, John F., and Timothy G. Dinan. "Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour." Nature reviews neuroscience 13.10 (2012): 701-712.

10

175

Gut-Microbiota-Brain Communication

- Bidirectional communication
  - Central nervous system (brain and spinal cord)
  - Autonomic nervous system (sympathetic and parasympathetic)
  - Enteric nervous system (intrinsic nervous system of GI tract)
  - Hypothalamic pituitary adrenal axis (HPA)
  - Microbiome (collection of microorganisms and their genomes in the gut)

Clarkea, Gerard, Ted Dinanb, and John Cryanc. "Microbiome-Gut-Brain Axis." (2013).

11

176

Vagus Nerve

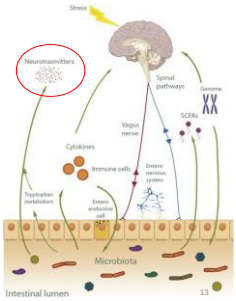
- Major nerve of the parasympathetic division of the autonomic nervous system
- Important pathway for bidirectional communication between the gut microbes and the brain
- Preclinical/animal studies demonstrate that probiotic effects on brain are dependent on vagal afferent signals
  - *Lactobacillus rhamnosus* directly activates vagal neurons
  - Induces region-dependent alterations in GABA receptor expression in the brain and reduced stress-induced corticosterone and anxiety- and depression-like symptoms via vagus nerve signaling in mice
- Vagotomized mice do not exhibit this effect

Bravo, Javier A., et al. *Proceedings of the National Academy of Sciences* 108.38 (2011): 16050-16055. 12

177

Neurotransmitters

- Acetylcholine
- Noradrenaline
- Adrenaline
- Gamma-amino butyric acid (GABA)
- Serotonin



Burokas et al., Advances in Applied Microbiology. 91 (2015): 1-62 13

178

Neurotransmitters & GI Function

Neurotransmitter	Released By	Function
GABA	Central Nervous System (CNS)	Relaxes lower esophageal sphincter
Norepinephrine	CNS, spinal cord, sympathetic nerves	Decreases motility, increased contraction of sphincters, inhibits secretions
Acetylcholine	CNS, autonomic system, other tissues	Increases motility, relaxes sphincters, stimulates secretion
Serotonin	GI tract, spinal cord	Facilitates secretion and peristalsis

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Bacteria & Neurotransmitters

Neurotransmitter	Genus
GABA	*Lactobacillus, Bifidobacterium*
Norepinephrine	*Escherichia, Bacillus, Saccharomyces*
Acetylcholine	*Lactobacillus*
Serotonin	*Candida, Streptococccus, Escherichia, Enterococcus*

Lyte, Mark. *Bioessays* 33.8 (2011): 574-581. 15

180

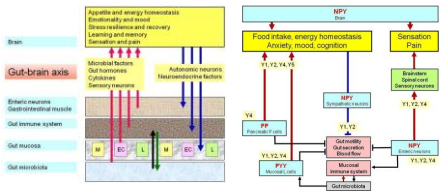
Serotonin

- Biogenic amine that functions as a neurotransmitter
  - Tryptophan is precursor
  - Involved in GI secretion
  - Gut motility
  - Pain perception
  - Maintenance of mood and cognition
- 95% of serotonin is contained in the gut in the mucosa and nerve terminals of the enteric nervous system
- Alterations in serotonin transmission may underlie pathological symptoms
  - Selective serotonin reuptake inhibitors are known to modulate psychiatric and GI disorders (e.g., IBS)

O'Mahony, S. M., et al. Behavioural brain research 277 (2015): 32-48. 16

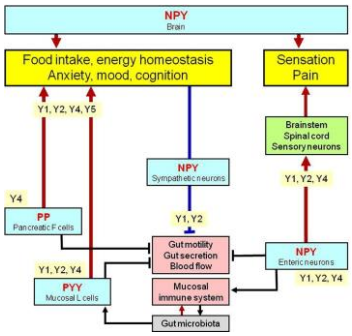
181

Gut Hormones and Neuropeptides



Holzer, Peter, Florian Reichmann, and Attila Farzi. "Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut–brain axis." *Neuropeptides* 46.6 (2012): 261-274. 17

182



184

Gut Hormones and Neuropeptides

Gut can communicate with the brain via hormonal signaling: gut peptides from enteroendocrine cells can act directly on the brain.

Germ-free studies suggest that the gut microbiota mediates and regulates the release of gut peptides

Forsythe, Paul, and Wolfgang A. Kunze. *Cellular and molecular life sciences* 70.1 (2013): 55-69.
Cameron, Jamieson, and Eric Doucet. *Applied Physiology, Nutrition, and Metabolism* 32.2 (2007): 177-189.
Schéle, Erik, et al. *Endocrinology* 154.10 (2013): 3643-3651.
20

185

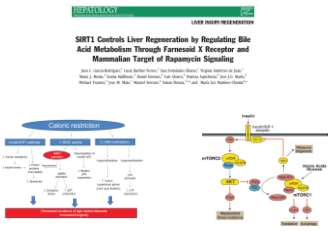
Interventions: epigenetic active foods,fasting, Probiotika, Prebiotika, mi RNAs

188

Caloric restriction: longevity in many models

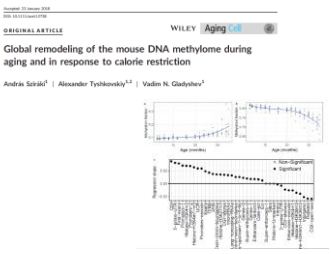
189

Fasting pathways: Sirt, mTOR pathways



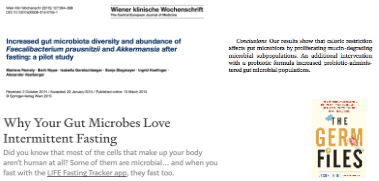
190

Caloric restriction and aging change epigenetic CpG -methylation structure



191

Fasting and Microbiota



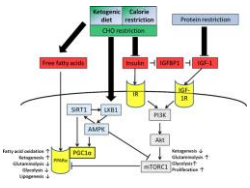
192

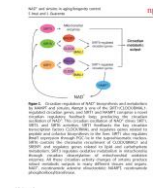
Caloric restriction- longevity fasting mimetics and senolytics ?

http://longevitydiet.info/images/TLD_Cover.jpg

193

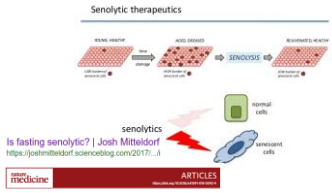
Caloric restriction, ketogenic diet involve SIRTs (+NAD, clock genes) + mTOR pathways (Metformin). What do fasting mimetics?





194

Caloric restriction: Rejuvenetion by senolysis? role for autophagy ?



83rd ICREA Colloquium, 2018

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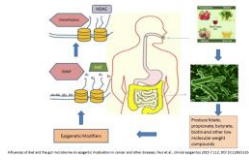
Effect of Plant Ingredient and Diet on Microbiota and Metabolites



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Probiotic

- Positive effects on health already 100 years ago suggested by Nobel Prize winner Elie Metchnikoff [Metchnikoff, 2004]
- Definition: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [FAO/WHO, 2002]
- Over 8000 research articles published since 2002 → several probiotic products on the market [Hill et al., 2014]
- Cell components of probiotics able to induce effects in host [Dotan and Rachmilewitz, 2005] but requirement for survivable cells remains a crucial factor for efficacy [Ma et al., 2004]

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Antimicrobial substances

- Probiotics produce various antimicrobial acting substances
- Examples: lactic acid, hydrogen peroxide, microcines, deconjugated bile acids [Oelschlaeger, 2010], bacteriocins [Maqueda et al., 2008]
- Antibiotics also produced by probiotics → reuterin:
  - Broad-spectrum antibiotic
  - Active against yeast, gram-positive and gram-negative bacteria, fungi, viruses, protozoa
  - Produced by strain ATCC55730 from L. reuteri [Cleusix et al., 2007]

198

Species

- Lactobacilli:
  - Present in GIT, oral cavity and vagina of humans [Walter, 2008]
  - Widespread use in production and fermentation of foods → ability to convert hexose sugars to lactic acid → preservation [Fijan, 2014]
  - Excellent for use as probiotics: high tolerance to acid and bile, capability to adhere to intestinal surfaces [Tulumoglu et al., 2013]
- Bifidobacteria:
  - First colonizers of the human gut together with lactobacilli [Turroni et al., 2012]
  - Well known for resistance against bile salts [Fijan, 2014]

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Species

- Bacillus species:
  - Either spore-forming aerobic or facultative aerobic, gram positive bacteria
  - B. subtilis, B. cereus, B. coagulans are members with probiotic characteristics [Fijan, 2014]
- Eschericha coli Nissle 1917:
  - Able to colonize the gut and compete with resident and pathogenic bacteria through multiple fitness factors [Behnsen et al., 2013]
  - Stimulation of epithelial defensin production → restoration of disturbed gut barrier
  - „Sealing effect" on tight junctions of enterocytes [Sonnenborn and Schulze, 2009]

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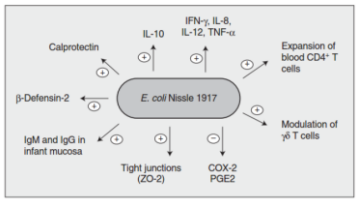

Figure 2. Various ways of immune modulation by E. coli Nissle 1917 (summary of data from in vitro and in vivo experiments) [Behnsen et al., 2013]

201

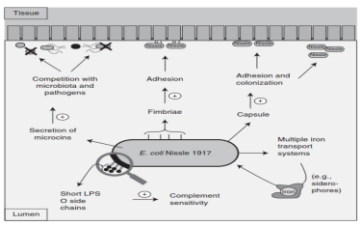

Figure 3. Multiple fitness factors possessed by E. coli Nissle 1917. Through these fitness factors E. coli Nissle 1917 is able to colonize the gut and compete with resident and pathogenic bacteria. [Behnsen et al., 2013]

202

Antibiotic associated diarrhea

- Antibiotic treatment → distrubance of GI flora
- Up to 30% of treated patients report symptoms like diarrhea [Barbut and Meynard, 2002] [McFarland, 1998]
- Especially infection with C. difficile is very serious → pseudomembranous colitis [Poutanen, 2004]

203

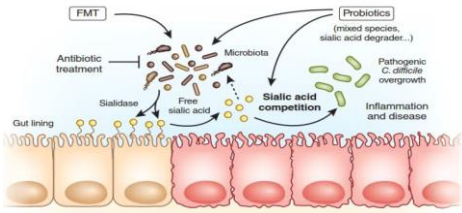

Figure 4. Competition for sialic acid after antibiotic treatment [Ley, 2014]

204

Treatment of antibiotic associated diarrhea with probiotics - meta-analyses

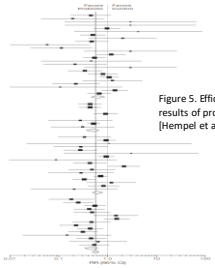

Figure 5. Efficacy results of probiotics [Hempel et al., 2012]

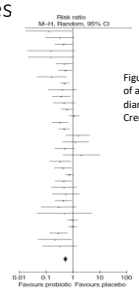

Figure 6. Developement of antibiotic associated diarrhea [Videlock and Cremonini, 2012]

205

Crohn‘s disease

- Intermittent transmural inflammation
- Can affect any segment of GIT, from mouth to anus [Fow and Grossman, 2007]
- Abdominal pain, diarrhea (bloody), fever, weight loss, signs of bowel obstruction [Baumgart and Sandborn, 2012]
- Recurrent flare-ups of symptoms [Biancone et al., 2003]
- Endogenous bacterial flora of patients can trigger cascade that results in intestinal injury → inflammatory mediators like lipopolysaccharides [Sartor, 2003]

206

Treatment of Crohn‘s disease with probiotics – meta-analyses

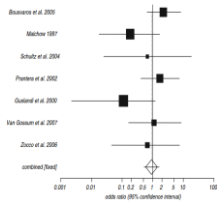

Figure 7. Clinical Relapse of Crohn’s disease considering probiotic therapy [Rahimi et al., 2008]

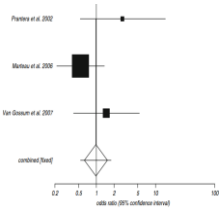

Figure 8. Endoscopic Relapse of Crohn’s disease considering probiotic therapy [Rahimi et al., 2008]

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Treatment of Crohn‘s disease with probiotics – meta-analyses

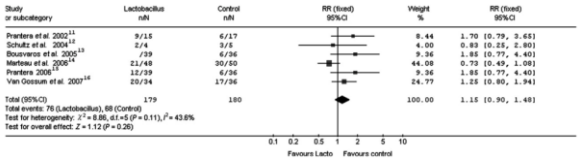

Figure 9. Relative risk for clinical relapse in patients treated with lactobacilli compared to placebo. [Shen et al., 2009]

208

Acute diarrhea

- One of most common GI disorders → high economic impact
- Characterized by defecating three or more loose stools per day
- Cause: viruses, bacteria, parasites
- In most cases self-limiting → no antibiotic treatment needed
- Treatment: replacement of fluid → reduces risk of dehydration but no shorter duration of diarrhea and vomiting → probiotics?! [Salari et al., 2012]

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Treatment of acute diarrhea with probiotics – meta-analyses

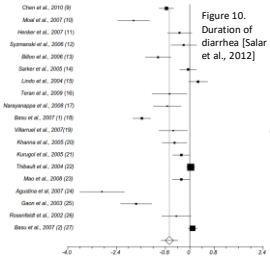

Figure 10. Duration of diarrhea [Salar et al., 2012]

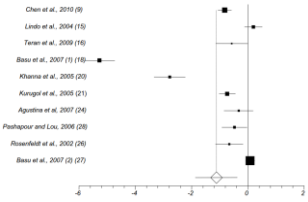

Figure 11. Duration of hospitalization [Salari et al., 2012]

210

Ulcerative colitis

- Continous area of inflammation without segments of normal tissue
- Typically affects mucosa of colon and rectum [Head and Jurenka, 2003]
- Exact etiology is unknown → genetic and environmental factors may be contributing [Thompson and Lees, 2011][Frolkis et al., 2013]
- Inflammation more frequent in areas with highest bacterial concentration
- Mucosal ulcers invaded by enteric bacteria → formation of fistula [Rahimi et al., 2008]

211

Treatment of ulcerative colitis with probiotics – meta-analyses

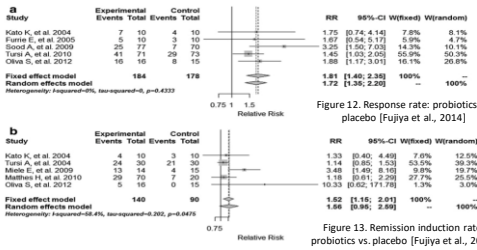

Figure 12. Response rate: probiotics vs. placebo [Fujiya et al., 2014]

Figure 13. Remission induction rate: probiotics vs. placebo [Fujiya et al., 2014]

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Treatment of ulcerative colitis with probiotics – meta-analyses

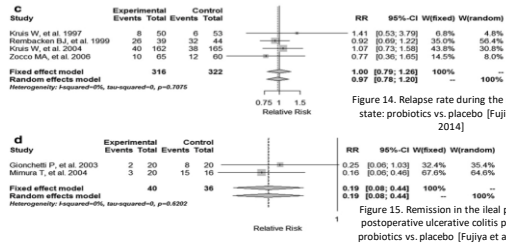

Figure 14. Relapse rate during the remission state: probiotics vs. placebo [Fujiya et al., 2014]

Figure 15. Remission in the ileal pouch in postoperative ulcerative colitis patients: probiotics vs. placebo [Fujiya et al., 2014]

213

Irritable bowel syndrome

- Chronic illness with severe impact on the life and its quality [Mönnikes, 2011]
- Characterized by altered bowel habits combined with unexplained abdominal pain, discomfort, bloating [Andresen and Camilleri, 2006]
- Affects about 3-15% of the general population [Cremonini and Talley, 2005]
- Risk factors: female gender, acute GI infections (Salmonella, Campylobacter), psychological factors [Ruigomez et al., 2007][Spiller, 2007]
- Alteration in intestinal microflora is common [Lin, 2004][Malinen et al., 2005]

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Treatment of irritable bowel syndrome with probiotics – meta-analyses

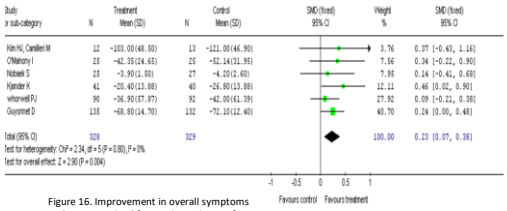

Figure 16. Improvement in overall symptoms (continous data) [Hoveyda et al., 2009]

215

Acute Pancreatitis

- Mild to severe inflammation of the pancreas [Forsmark and Baillie, 2007]
- Can lead to necrosis of the pancreas → major cause of morbidity and mortality in patients [UK Working Party on Acute Pancreatitis, 2005]
- Small bowel bacterial overgrowth and subsequent bacterial translocation → late infections → cause of death in patients with acute necrotizing pancreatitis [Besselink et al., 2004]
- Antibiotics used widely, but no significant benefits for patients with necrotizing acute pancreatitis [Dellinger et al., 2007] [Isenmann et al., 2004] [Mazaki et al., 2006]

216

Treatment of acute pancreatitis with probiotics – meta-analyses

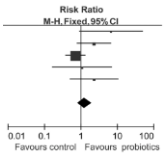

Figure 17. Infection of necrotic pancreas tissue [Gou et al., 2014]

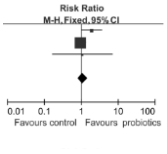

Figure 18. Total infection [Gou et al., 2014]

217

Treatment of acute pancreatitis with probiotics – meta-analyses

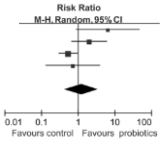

Figure 19. Operation [Gou et al., 2014]

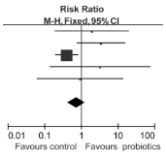

Figure 20. Mortality [Gou et al., 2014]

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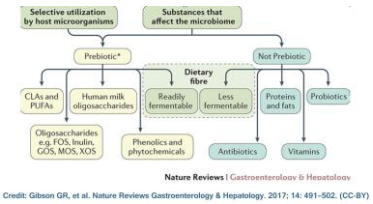
Prebiotics what is it?



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Postbiotics

- **Bacteriocins** *(protective compounds that make life hard for the bad guys)**
- **Enzymes** *(help to digest food, get rid of toxins and assist other metabolic processes)**
- **Vitamins** *(like the B's and vitamin K)**
- **Amino acids** *(building blocks of protein)**
- **Neurotransmitters** *(carry messages between the nerves and brain and can even affect appetite)**
- **Immune-signaling compounds** *(they support the body's immune cells)**
- **Short-chain fatty acids** *(created from fiber, they keep the intestinal lining strong and healthy)**
- **Nitric oxide** *(crucial for cardiovascular health)**
- **Organic acids** *(such as Fulvic and Humic acid. They combine with minerals, making them easier to absorb and help maintain the correct pH in the GI tract)**

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Fermengtation

221

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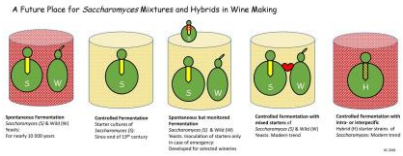
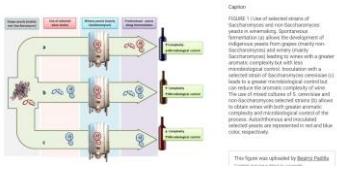
Fermentation spontaneous strater cultures



222

Fermentation, wine



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Fermented Food Product

- Bread
- Idli
- Natto
- Kimchi
- Kombucha
- Sauerkraut
- Miso

224

Bread

- Bread is one of the oldest prepared foods. Evidence from 30,000 years ago in Europe revealed starch residue on rocks used for pounding plants.
- Bread is a staple food prepared from a dough of flour and water, usually by baking.
- Bread is served in various forms with any meal of the day.
- Nutritionally, bread is known as an ample source for the grains category of nutrition.
- maximizes CO_2 production, which leavens bread.
- other microbes used to make special breads (e.g., sourdough bread).
- can be spoiled by *Bacillus* species that produce ropiness.

225

Idli

- Idli is a traditional breakfast in South Indian households, especially in Andhra Pradesh, Karnataka, Tamil Nadu and Telangana where it is a popular breakfast dish that is consumed in numerous households.
- The cakes are made by steaming a batter consisting of fermented black lentils (de-husked) and rice.
- In idli made with a 1:1 ratio of black gram to rice, batter volume increased about 47 percent 12 to 15 hours after incubation at 30°C.
- Using a 1:2 ratio of black gram to rice, batter volume increased 113 percent and acidity rose to 2.2 percent in 20 hours at 29°C.

226

Sauerkraut

- Sauerkraut is finely cut cabbage that has been fermented by various lactic acid bacteria.
- It has a long shelf life and a distinctive sour flavour, both of which result from the lactic acid that forms when the bacteria ferment the sugars in the cabbage.
- Fermentation by lactobacilli is introduced naturally, as these air-borne bacteria culture on raw cabbage leaves where they grow.
- Sauerkraut is made by a process of pickling called lactic acid fermentation that is analogous to how traditional (not heat-treated) pickled cucumbers and kimchi are made.

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Kimchi

- Kimchi, is a traditional side dish made from salted and fermented vegetables, most commonly napa cabbage and Korean radishes, with a variety of seasonings including chili powder, scallions, garlic, ginger, and *jeotgal*.
- Kimchi is a traditional Korean dish consisting of pickled vegetables, which is mainly served as a side dish with every meal, but also can be served as a main dish.
- Kimchi has been a staple in Korean culture, but historical versions were not a spicy dish.

228

Natto

- Nattō is a traditional Japanese food made from soybeans fermented with *Bacillus subtilis var. natto*.
- Nattō is made from soybeans, typically nattō soybeans. Smaller beans are preferred, as the fermentation process will be able to reach the center of the bean more easily.
- Some eat it as a breakfast food.
- It is served with soy sauce, karashi mustard and Japanese bunching onion.
- Nattō may be an acquired taste because of its powerful smell, strong flavor, and slimy texture.

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Fermentation, postbiotic and metabolic syndrome


Targeting gut microbiota with a complex mix of dietary fibers improves metabolic diseases

Patrice D. Cani

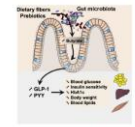

Figure 1| Targeted modulation of the gut microbiota improves metabolism. Dietary fibers and prebiotics are fermented by the gut microbiota into short chain fatty acids (SCFAs) such as butyrate. SCFAs are able to stimulate the secretion of both glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) by the enteroendocrine L-cells. GLP-1 increases glucose utilization and insulin sensitivity and ultimately contributes to reductions in hemoglobin A1c (HbA1c). In addition, PYY and GLP-1 promote weight loss and reduce plasma lipids. Altogether, these mechanisms partially explain the beneficial effects observed with increased intake of dietary fibers and prebiotics.

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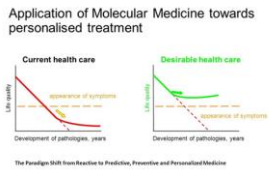
From intervention to a preventive, personalised health care



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