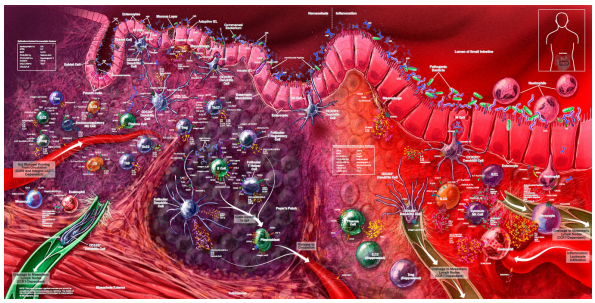
Bioinformatics Workshop – SPR2022

Overview of the Gut Microbiota

Daniel Bergey Ph.D. // Great Basin College



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Brief Outline

- ❑ The role of microbiota in health
- ❑ Brief anatomy of gut mucosa
- ❑ Microbiota-immune system connection
- ❑ Maintenance of gut immune homeostasis
- ❑ Inflammation and gut microbiota
- ❑ Intro to microbiota-gut-brain axis

2

Microbiome: A community of micro-organisms living together in a particular habitat.

- ❑ Every human body surface is exposed to the environment, and every body part with an opening to the environment, has a *unique microbiome.*
- ❑ The human gastrointestinal tract harbors a unique microbiota community of about **10^{14} total microbes** consisting mainly of bacteria, but also archaea, viruses, fungi, and protozoa. **1,500-2,000** different bacterial species.
- ❑ Gut microbiota are highly variable among individuals, but certain common combinations of bacteria are found in healthy individuals.
- ❑ **Diet** appears to be the primary factor in shaping the gut microbiota across during an individual's lifetime.
- ❑ Intestinal bacteria play a crucial role in maintaining immune and metabolic homeostasis, protection against pathogens, *and neurological development*

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5433529/

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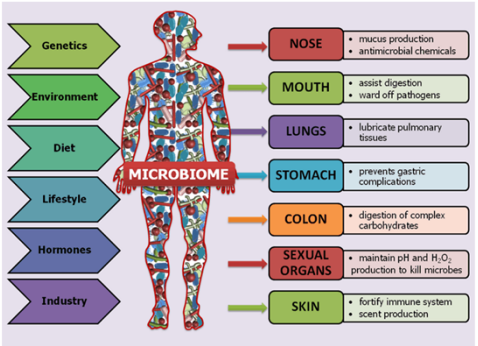
Various Microbiomes Living Within and On a Healthy Human



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A Short Story…

→ ***One day some key body parts were arguing with each other about who was <u>most</u> important:***

- **The brain** said: "I'm the most important since I control everything".
- **The heart** said: "I'm the most important since my beating keeps the body going".
- **The liver** said: "I'm the most important since I play diverse roles in metabolism, excretion, detoxication, immune response, etc."
- Finally, **the GUT** said: "NO, I'm the most important! I digest, absorb, protect, defend, excrete, produce hormones, etc."
- **EVERYONE LAUGHED UNCONTROLLABLY --- *HA HA HA !!***
- The GUT got angry and shut down
- Within a few days all the other body parts agreed... ***<u>Gut</u> <u>IS Most</u> <u>Important</u> !***

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"All disease begins in the gut."

*- **Hippocrates 460 BC – 370 BC***

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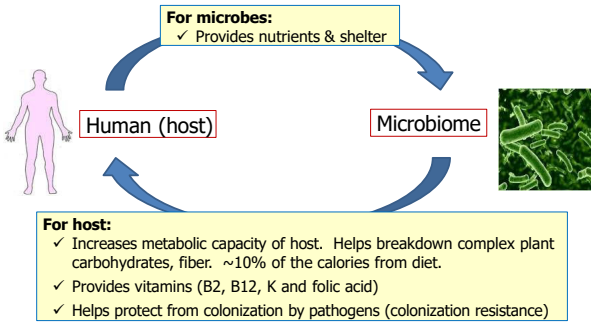
Gut Microbiota in Health - Symbiosis

- **Symbiotic relationship: Interaction between two different organisms living in close physical association, typically to the advantage of both.**



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The GI Tract Presents Unique Challenges for the Mammalian Immune System

- The GI tract (or GUT) <u>must tolerate</u> the presence of an estimated **100 trillion** luminal microbes (microbiota), and NOT respond <u>inappropriately</u> to these foreign squatters or their products, but still <u>protect</u> the intestinal mucosa from potentially harmful dietary antigens and invading pathogens.
- The **intestinal epithelium** is a <u>single layer</u> of cells lining the gut that is essential for preserving gut homeostasis, and acts both as a <u>physical barrier</u>, and a <u>coordinating hub</u> for immune defense and crosstalk between bacteria and immune cells.

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<u>Metagenomics</u> methods can currently map about 90% of microorganism genomes, which means these genomes can be <u>prioritized</u> for analysis.

- Based on recent large-scale metagenomic studies of the microbiome from different body sites in individuals with both Westernized and non-Westernized lifestyles, the diversity of the human microbiome is estimated at 25 different phyla, an average of 2,000 genera and 5,000 species, and 316 million genes.

  However, the diversity of a <u>large fraction</u> of the human microbiome remains unknown and unexplored.

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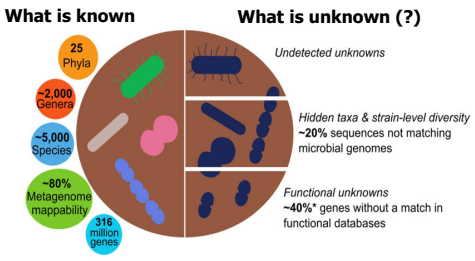
The Human Microbiome



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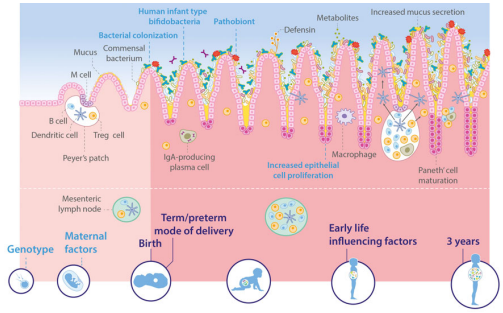
Normal Gut Microbiota & Immune Development

- Early life nutrition is a critical factor in <u>shaping</u> the gut microbiota, and promoting appropriate <u>brain development</u>. Gaining better understanding of the microbiota-gut-brain relationship, and the role of nutrition in early neurological development is a very active area of current research.



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Commensal Microbes Provide a <u>Protective</u> Function (barrier effect)

- Compete and adhere to potential <u>attachment sites</u> on the brush border of intestinal epithelial.
- Compete for available nutrients.
- Produce <u>antimicrobial</u> compounds (defensins, bacteriocins).

→ ***Collectively these factors (and the mucous layer) help prevent attachment and entry of pathogenic bacteria into intestinal epithelial cells***

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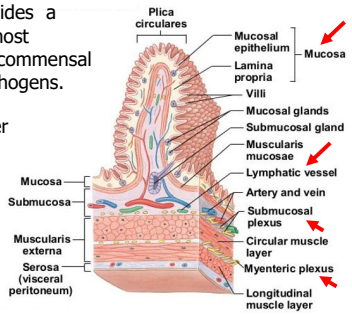
Microanatomy of Gut Wall

- **Intestinal mucosa** provides a physiological barrier for most microbes, including both commensal bacteria and invading pathogens.
- The **lamina propria** layer contains various immune cells including DCs, Macs, IgA-producing plasma cells, B cells and various T cells.
- Note the <u>extensive innervation</u> of the gut (referred to as "the second brain")



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<u>Enteric</u> Nervous System

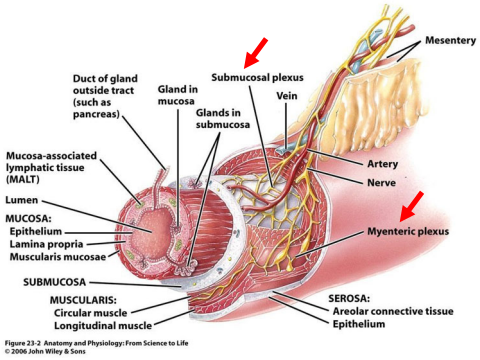

Figure 23-2 Anatomy and Physiology: From Science to Life
© 2006 John Wiley & Sons

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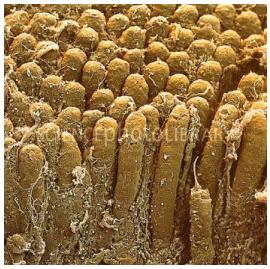
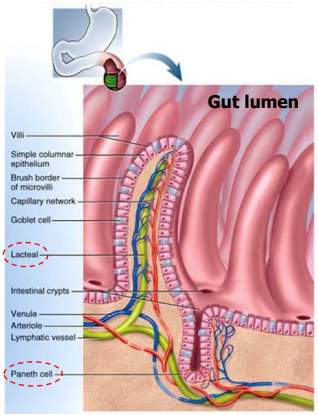
Intestinal Villi and Lymph/Blood Circulation





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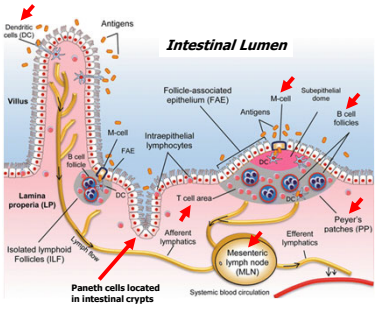
GALT: Gut-Associated Lymphoid Tissue

- **GALT** is the largest lymphatic organ in the human body and contains about ~**75%** of the body's total lymphocytes.
- GALT is exposed to more antigens than any other part of the body (e.g., commensal bacteria, dietary gut antigens, and invasive pathogens).



https://link.springer.com/chapter/10.1007/978-3-319-28609-9_14

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Major Components of GALT

- **Peyer's patches (PP)** are a collection of lymphoid nodules distributed in the mucosa and submucosa of the intestine. Contain various immune cells (B and T cells, macrophages, DCs).
- Follicle-associated epithelium **(FAE)** is a single layer of epithelial cells separating lymphoid areas of PP from the intestinal lumen.
- **M cells** are embedded in the epithelium (FAE) just above Peyer's Patches; the "gateway" for transport of luminal antigens to PP.
- **Paneth cells** secrete antimicrobial proteins into gut lumen.
- **Dendritic cells (DC)** sample gut lumen contents; key immune sensors and regulators (e.g., mediate IgA production).
- **IgA antibodies** are secreted into gut lumen.
- **Mesenteric lymph nodes (MLN)** collect lymph from intestinal mucosa.

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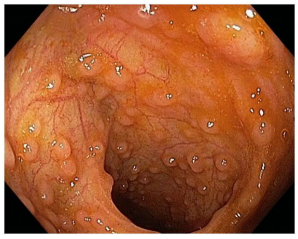
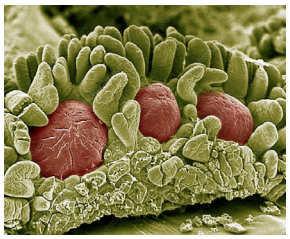
Peyer's Patches
("Intestinal Tonsils")

Peyer's patches (PP) in the distal ileum (20-yr old man).



Scanning EM of Peyer's patches (colorized)



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Role of DCs and IgA in Gut Immune Surveillance & Tolerance

- Dendritic cells (DC) continuously sample luminal antigens and *promote tolerance* to microbiota by inducing **IgA** synthesis in B cells, and mediating differentiation of **Treg** cells. Functional effectiveness of intestinal DCs is strongly influenced by the gut microenvironment, including the presence of commensal bacterial metabolites, epithelial cell-derived factors, and dietary products.
- DCs interact with luminal antigens that cross **M cells** of **Peyer's patches** (PP), and from isolated lymphoid follicles (ILF) that are transported into the lamina propria (LP).
- DCs also sample antigens directly from the lumen via trans-epithelial projections, and then either (a) present antigens to local lymphocytes, OR (b) migrate to mesenteric lymph nodes **(MLN)** for lymphocyte priming, resulting in subsequent systemic immune activation.

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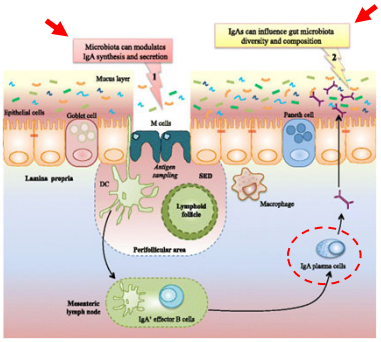
Role of DCs and IgA in Gut Immune Surveillance & Tolerance



https://www.researchgate.net/publication/294284585_Policing_of_gut_microbiota_by_the_adaptive_immune_system

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Role of IgA in Gut Immunity

- Under homeostatic conditions, immunoglobulin A **(IgA)** is the major immunoglobulin isotype in the intestinal mucosa (and all mucosa). **2-3 grams** of IgA is produced and secreted into the gut lumen every day
- Gut microbes trapped by **DCs** stimulate the production of **IgA** in **Peyer's patches** (or the mesenteric lymph nodes).
- Subsequent IgA binding to microbes, or their products, inhibits bacterial translocation across the gut epithelium, and neutralizes toxins at the intestinal mucosal surface.

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T-regulatory (Treg) Cells and Tolerance to Commensal Microbes

- "Germ-free" (GF) animal models (e.g., mice) provided the first formal proof that the microbiota was required for the induction and maintenance of intestinal **Treg** cells.
- Mice, and humans, treated with antibiotics show depletion in resident microbiota, correlating with a drastic reduction in the intestinal population of Treg cells.
- In addition to known immune-related roles, recent studies show that intestinal **Treg** cells also exert important non-immune functions in the gut, such as promoting local tissue repair and preserving the integrity of the intestinal epithelial barrier.

https://www.frontiersin.org/articles/10.3389/fimmu.2020.600973/full

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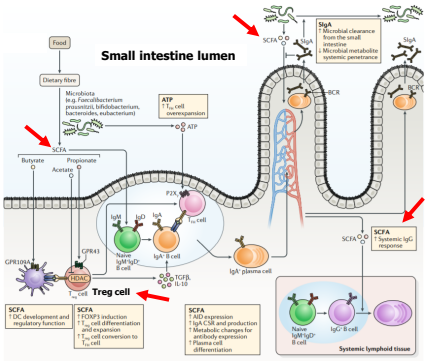
- **Commensal Gut Microbes Generate Short Chain Fatty Acids (SCFAs) that Induce Treg Cell Differentiation**



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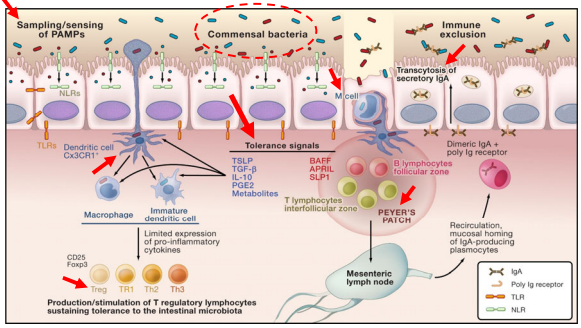
Innate & Adaptive Immune Responses in Gut Surveillance & Tolerance to Microbiota

- Potential tissue-damaging, inappropriate T cell responses can be inhibited by immunosuppressive cytokines and regulatory T cells (T regs).



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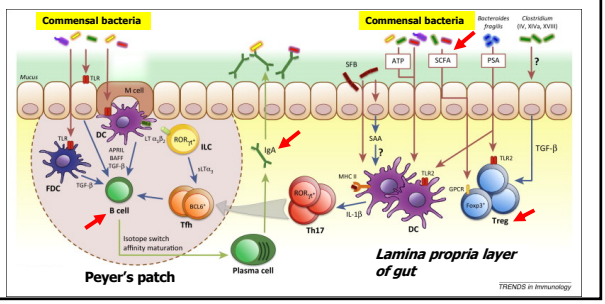
Role of Microbiota and Immune Response in Maintaining Gut Homeostasis

- Microbiota stimulation leads to B cell switch to IgA, induction of Treg cells, and T cell differentiation to Th17
- SCFA (short chain fatty acids); PSA (polysaccharide A); SFB: segmented filamentous bacteria



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Key Immune Signals and Receptors

- **Cytokines:** Signal molecules that regulate hematopoiesis and host responses to infection, immune responses, inflammation, and trauma. Some ae pro-inflammatory, and others reduce inflammation and promote healing.
- **PRRs** (pattern recognition receptors): Innate system component consisting of large family of receptors in host cells that recognize evolutionarily conserved molecular structures found on general classes pathogens (e.g., TLR).
- **PAMPs** (pathogen-associated molecular patterns): Pathogen-derived signal molecules (e.g., LPS, peptidoglycan) recognized by PRRs that elicit immune response.
- **DAMPs** (danger/damage-associated molecular patterns): Host-derived signal molecules that initiate immunes responses. *Can also promote pathological inflammatory responses.*

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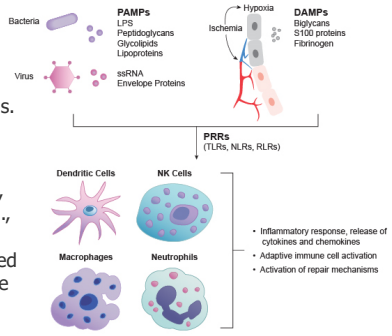
PAMPs & DAMPs Interact w/Host PRRs to Activate Innate Immunity & Inflammatory Responses

- Microbe-derived **PAMPs** drive inflammation in response to infections.
- **DAMPs:** derived from host cells including tumor cells, dead/dying cells (e.g., sterile inflammation) or substances released from cells in response to ***stress***, such as hypoxia.



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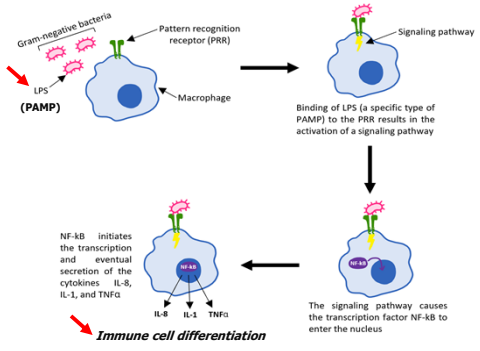
PAMP-PRR Interaction Triggers Immune Cell Activation and Cytokine Release



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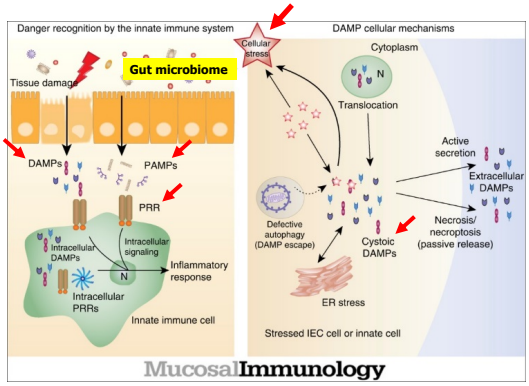
Gut Mucosal DAMPs & Inflammation



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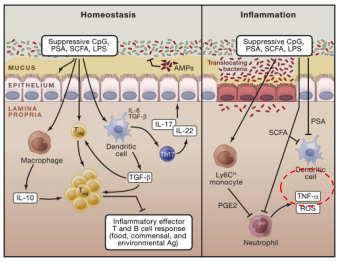
Gut Microbiota in Homeostasis and Inflammation

- **Left panel:** Commensal microbes promote induction of **Treg** cells via direct sensing of microbial products or metabolites by T cells or DCs. Other commensal microbes promote the induction of **Th17 cells** that can regulate function and homeostasis of gut epithelial cells.
- **Right panel:** Commensal-derived metabolites can have local and systemic effects on inflammatory cells. For example: ***SCFAs inhibit neutrophil activation.*** Upon entrance into tissue, inflammatory monocytes can respond to microbial products by producing immune signal molecules (e.g., PGE_2) that limit neutrophil activation and associated tissue damage.



- **SCFA: Short chain fatty acids**
- **AMP: Antimicrobial proteins**
- **PSA: Polysaccharide A**
- **LPS: Lipopolysaccharide**

https://www.cell.com/fulltext/S0092-8674(14)00345-6v

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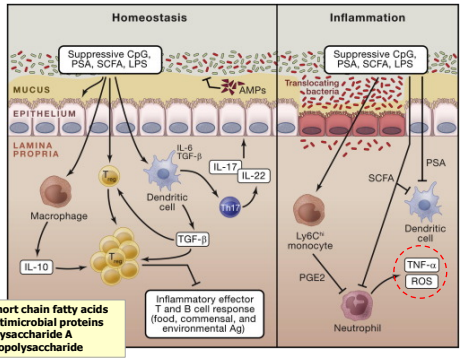
Gut Microbiota in Homeostasis & Inflammation



- **SCFA: Short chain fatty acids**
- **AMP: Antimicrobial proteins**
- **PSA: Polysaccharide A**
- **LPS: Lipopolysaccharide**

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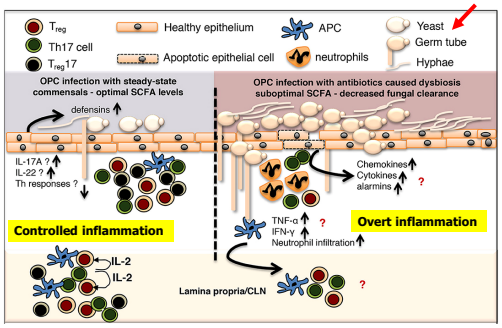
- **SCFAs contribute to immune homeostasis in mucosa, BUT… excessive & sub-optimal levels of SCFAs are often associated with inflammation and cancer.**

→ ***Example below: Response to oralpharyngeal candidiasis (OPC)***



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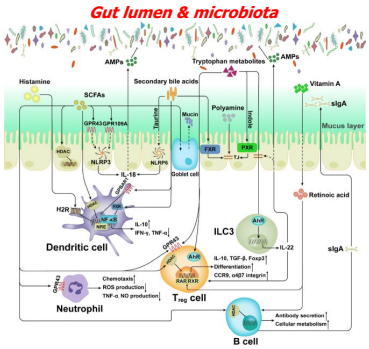
Metabolites Derived from Microbiota or Host Mediate Host-Microbiota Interactions

- **SCFAs** (short chain fatty acids)
- **AMPs** (antimicrobial peptides)
- **HADC** (histone deacetylase)
- **TJ** (tight junction)
- **AhR** (aryl hydrocarbon receptor)
- **FXR** (farnesoid X receptor)
- **PXR** (pregnane X receptor)
- **ILC3** (group 3 innate lymphoid cell)



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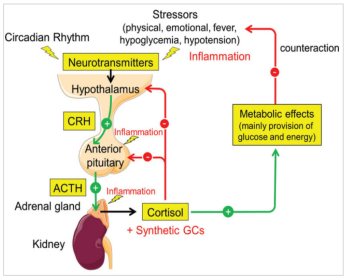
Inflammation and the HPA Axis
(HPA: hypothalamic-pituitary-adrenal axis)

- Activation of the HPA axis promotes inflammatory responses in both the brain and peripheral tissues.
- Depressed individuals have elevated levels of cortisol and pro-inflammatory cytokines, such as C-reactive protein (CRP) in their blood.
- HPA axis hyperactivity also promotes development of a range of cardiometabolic, inflammatory, endocrine, and neural disorders.



https://journals.lww.com/cmj/fulltext/2020/04050/gut_hormones_in_microbiota_gut_brain_cross_talk.11.aspx

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The HPA Axis (Summary)

- Various "stressors" (physical, emotional, fever, hypoglycemia, or hypotension) trigger release of corticotropin-releasing hormone **(CRH)** from the hypothalamus.
- CRH induces release of adrenocorticotropic hormone **(ACTH)** from the anterior pituitary, which in turn stimulates the adrenal gland to produce and release **cortisol.**
- Cortisol initiates diverse metabolic effects (e.g., mobilization of fuel molecules), which attempt to counteract the stressor. *Cortisol also directly suppresses immune system.*
- Regulation of the HPA axis is mediated by cortisol-dependent negative feedback on the hypothalamus and anterior pituitary. Synthetic glucocorticoids (GCs) also cause negative feedback regulation, but this can also lead to adrenal suppression.

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Is Cortisol Pro-inflammatory or Anti-inflammatory ?

- Virtually all cells in the body have cortisol receptors (glucocorticoid receptor; GR)
- Cortisol suppresses inflammation by inhibiting several pro-inflammatory cytokines produced by some immune cells
- Chronic stress leads to overproduction of cortisol. And over time, immune cells may become desensitized to cortisol and express fewer cortisol receptors. In this way, **chronic stress** can lead to **chronic inflammation** because the normal anti-inflammatory effect of cortisol is diminished.

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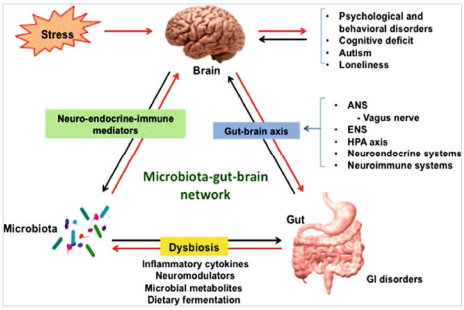
Microbiota-Gut-Brain Axis

- Stress increases norepinephrine levels, which can stimulate the proliferation of enteric pathogens. Additionally, the ANS is another pathway through which the CNS influences enteric microbiota. Parasympathetic and **vagal outputs** to the intestine and stomach are altered after acute stress stimulation.



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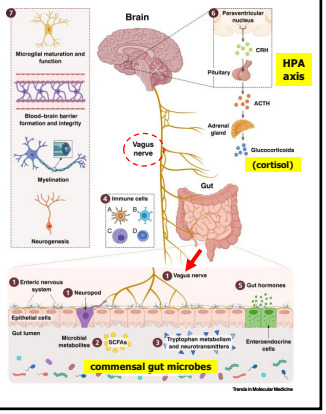
Microbiota-Gut-Brain Axis

- Recent research demonstrate as an important role for gut microbiota in the regulation of brain development and behavior.
- Host pattern-recognition receptors **(PRRs)** that recognize conserved microbe surface molecules (peptidoglycans, etc.) have emerged as key regulators of gut microbiota–brain interactions.

https://www.sciencedirect.com/science/article/abs/pii/S1471491420301325



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Microbiota-Gut-Brain Axis

- Studies on "germ-free" (GF) animals demonstrate that microbiota influence stress reactivity and stress/anxiety-like behavior, AND regulate the **set point for HPA activity** (sensitive !). GF animals exhibit lower overall anxiety but, when stressed, exhibit an increased stress response with highly elevated levels of **ACTH and cortisol (and CRP)**
- **Both neural (vagus) and hormonal (HPA axis)** lines of communication combine to enable brain to influence activities of diverse intestinal immune cells including epithelial cells, enteric neurons, smooth muscle cells, and enterochromaffin cells (produce epinephrine & norepinephrine). These cells, in turn, are under the influence of the **gut microbiota**, which impacts the brain–gut axis not only locally with intestinal cells, but also by *directly influencing* neuroendocrine and metabolic systems.

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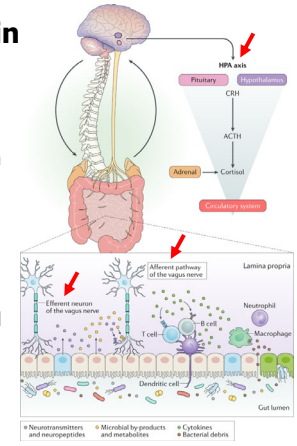
Microbiota-Gut-Brain Axis

- Two-way communication between the gut microbiota and the central nervous system (CNS) is mediated by several direct and indirect pathways of the gut–brain axis.
- Microbiota affects the expression of genes involved in HPA axis regulation and local metabolism of glucocorticoids in chronic psychosocial stress *(COVID lockdowns ?)*



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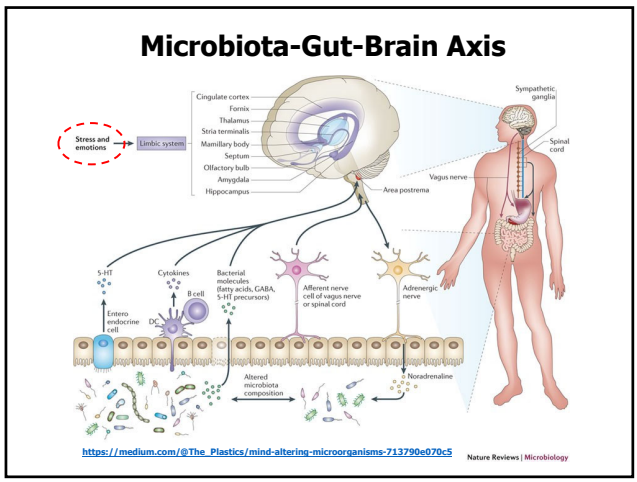
Microbiota-Gut-Brain Axis
Stress and emotions
Limbic system
Cingulate cortex
Fornix
Thalamus
Stria terminalis
Mamillary body
Septum
Olfactory bulb
Amygdala
Hippocampus
Area postrema
Sympathetic ganglia
Spinal cord
Vagus nerve
5-HT
Cytokines
B cell
Bacterial molecules (fatty acids, GABA, 5-HT precursors)
Afferent nerve cell of vagus nerve or spinal cord
Adrenergic nerve
Entero endocrine cell
DC
Noradrenaline
Altered microbiota composition
https://medium.com/@The_Plastics/mind-altering-microorganisms-713790e070c5
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