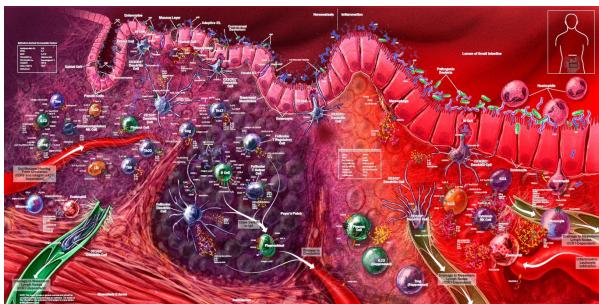


Bioinformatics Workshop – SPR2022
Overview of the Gut Microbiota
 Daniel Bergey Ph.D. // Great Basin College



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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

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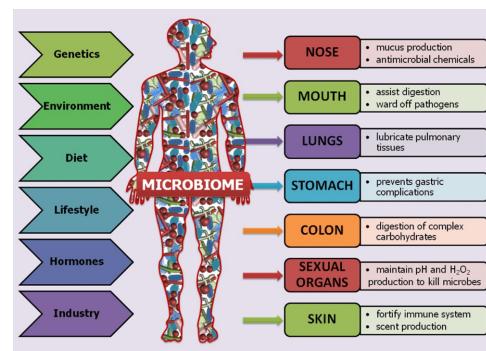
Microbiome: A community of micro-organisms living together in a particular habitat.

- ❑ 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 **10¹⁴ 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/gmc/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 most 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, detoxification, 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... **Gut IS Most Important!**

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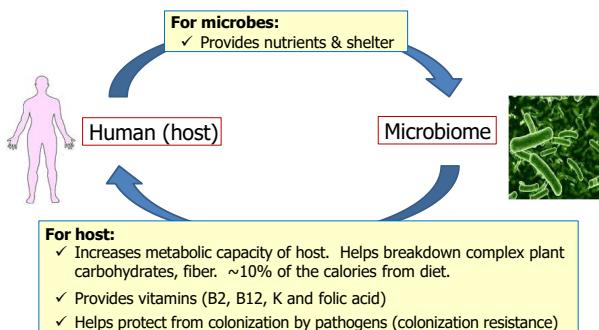
"All disease begins in the gut."

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

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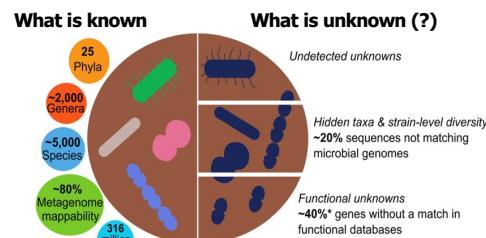
Metagenomics methods can currently map about 90% of microorganism genomes, which means these genomes can be prioritized for analysis.

- 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 large fraction of the human microbiome remains unknown and unexplored.

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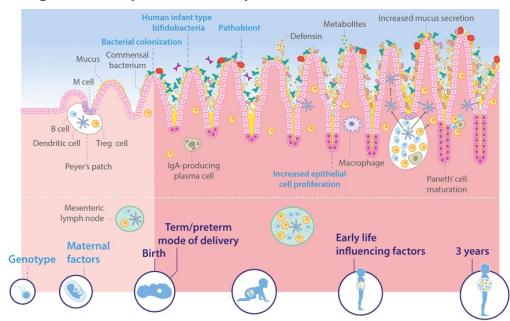
The Human Microbiome



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Normal Gut Microbiota & Immune Development

- Early life nutrition is a critical factor in shaping the gut microbiota, and promoting appropriate **brain development**. 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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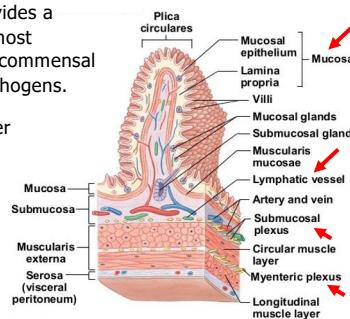
Commensal Microbes Provide a Protective Function (barrier effect)

- Compete and adhere to potential attachment sites on the brush border of intestinal epithelial.
 - Compete for available nutrients.
 - Produce antimicrobial 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 extensive innervation of the gut (referred to as "the second brain")



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Enteric Nervous System

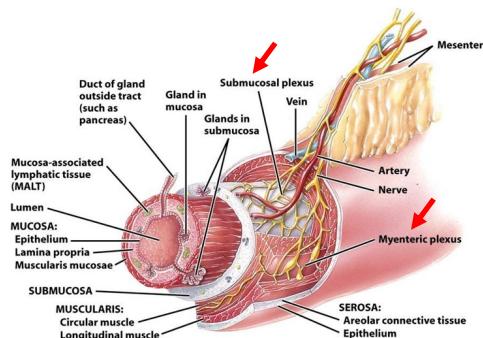
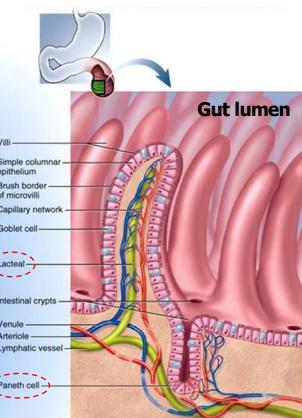


Figure 23-3 Anatomy and Physiology: From Science to Life

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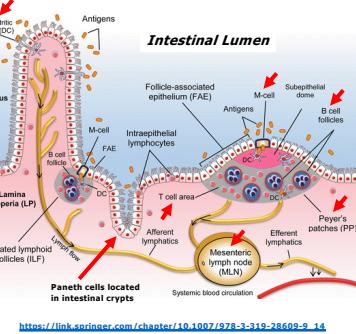
Intestinal Villi and Lymph/Blood Circulation



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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.

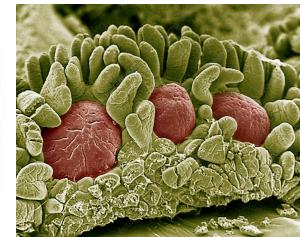
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Peyer's Patches ("Intestinal Tonsils")

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



Scanning EM of Peyer's patches (colorized)



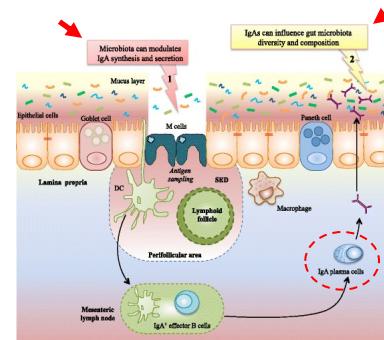
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Role of DCs and IgA in Gut Immune Surveillance & Tolerance

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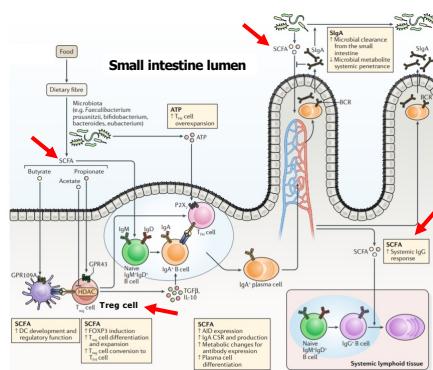
T-regulatory (Treg) Cells and Tolerance to Commensal Microbes

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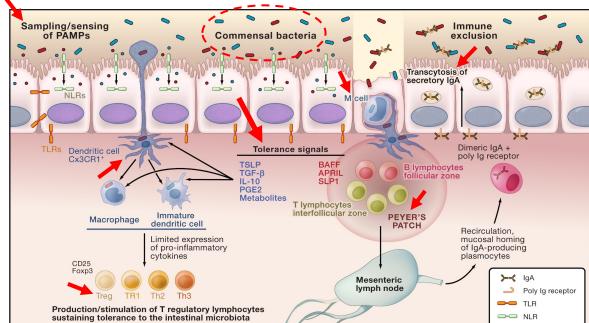
- Commensal Gut Microbes Generate Short Chain Fatty Acids (SCFAs) that Induce Treg Cell Differentiation



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

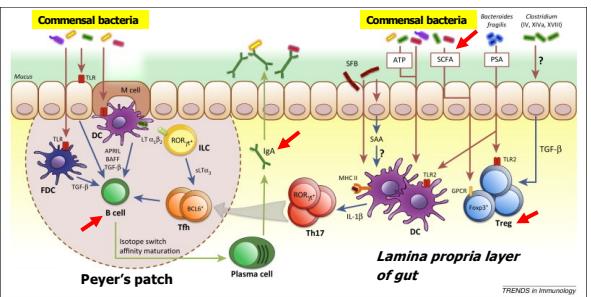
- Potential tissue-damaging, inappropriate T cell responses can be inhibited by immunosuppressive cytokines and regulatory T cells (T reg).



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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 are 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 immune responses. *Can also promote pathological inflammatory responses.*

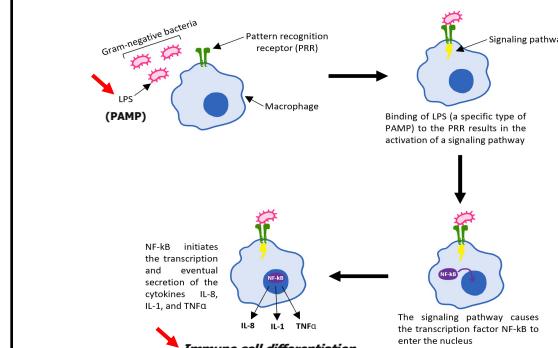
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PAMPs & DAMPs Interact w/Host PRRs to Activate Innate Immunity & Inflammatory Responses

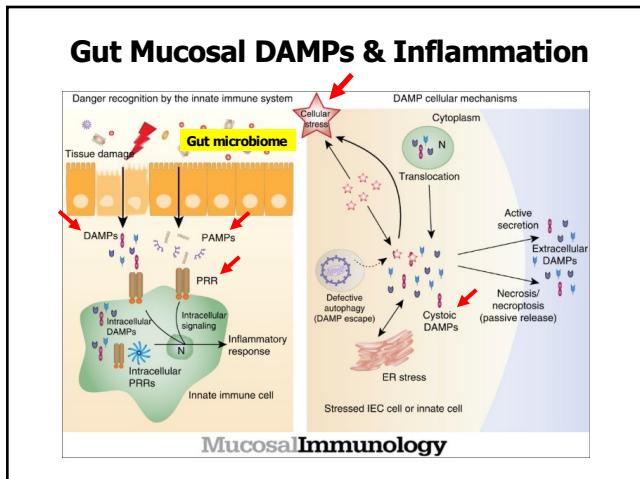
- Microbe-derived PAMPs** drive inflammation in response to infections.
 - DAMPs:** derived from host cells including tumor cells, dead/dying cells (e.g., sterile inflammation) or substances released from cells in response to **stress**, such as hypoxia.
- PAMPs**: LPS, Peptidoglycans, Glycolipids, Lipoproteins
DAMPs: Bigrans, S100 proteins, Nitric oxide
PRRs: TLRs, NLRs, RLRs
- Bacteria, Virus, Hypoxia, Ischemia, ssRNA, Envelope Proteins
- Dendritic Cells, NK Cells, Macrophages, Neutrophils
- Inflammatory response, release of cytokines and chemokines
 - Adaptive immune cell activation
 - Activation of repair mechanisms

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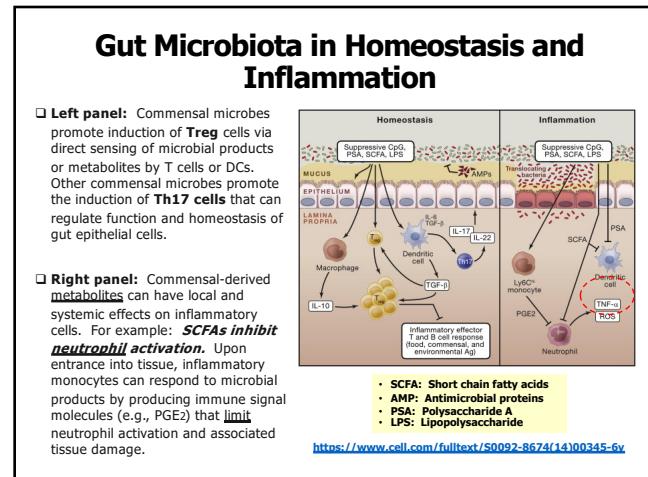
PAMP-PRR Interaction Triggers Immune Cell Activation and Cytokine Release



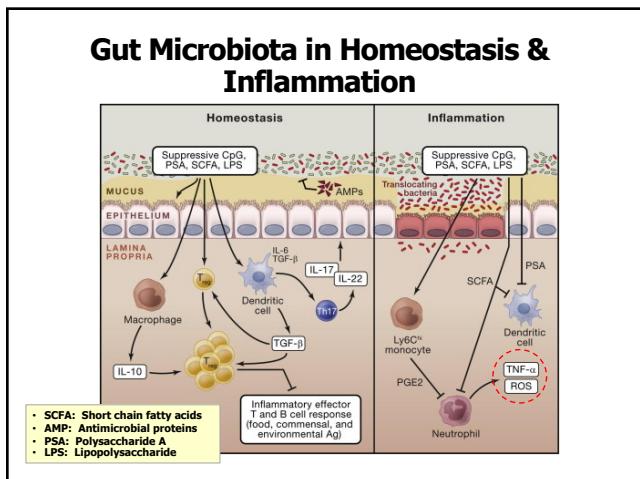
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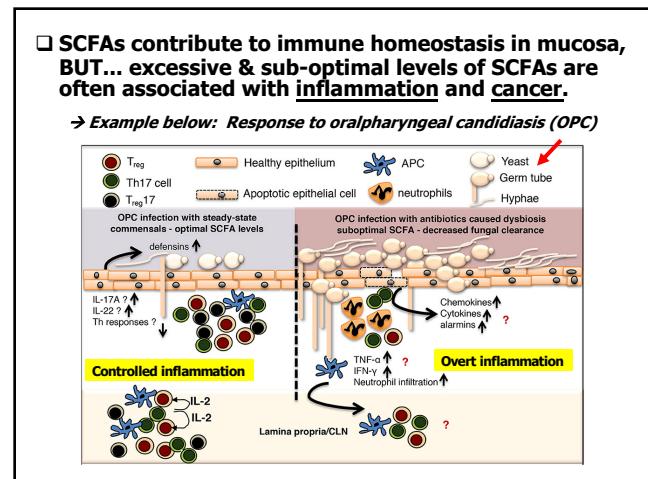
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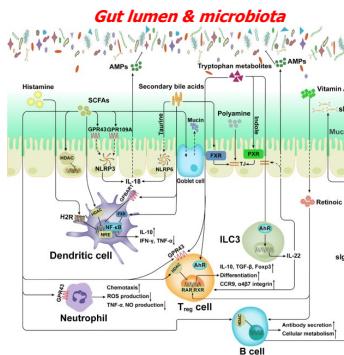


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Metabolites Derived from Microbiota or Host Mediate Host-Microbiota Interactions

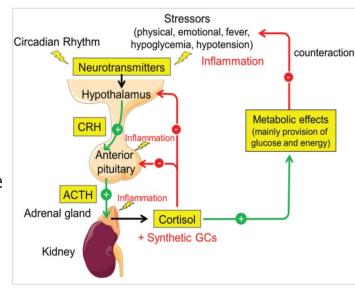
- ❑ **SCFAs** (short chain fatty acids)
- ❑ **AMPs** (antimicrobial peptides)
- ❑ **HADC** (histone deacetylase)
- ❑ **TJ** (tight junction)
- ❑ **AhR** (aryl hydrocarbon receptor)
- ❑ **FXR** (farnesoid X receptor)
- ❑ **PXR** (pregnane X receptor)
- ❑ **ILC3** (group 3 innate lymphoid cell)



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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.



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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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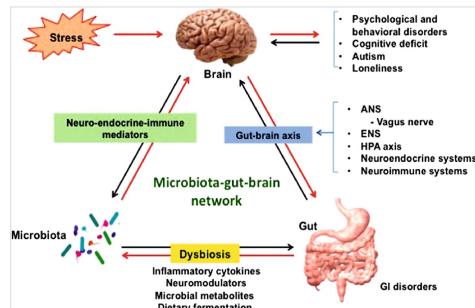
Is Cortisol Pro-inflammatory or Anti-inflammatory ?

- ❑ 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.

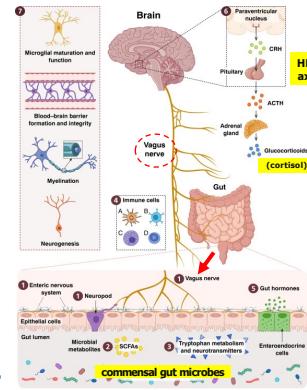


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Microbiota-Gut-Brain Axis

- Recent research demonstrate 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/S1743914020301375>



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Microbiota-Gut-Brain Axis

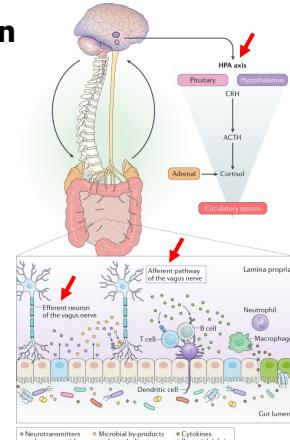
- Studies on "germ-free" (GF) animals demonstrate that microbiota influence stress reactivity and stress/anxiety-like behavior, AND regulate the set point for HPA activity (sensitive!). GF animals exhibit lower overall anxiety but, when stressed, exhibit an increased stress response with highly elevated 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.

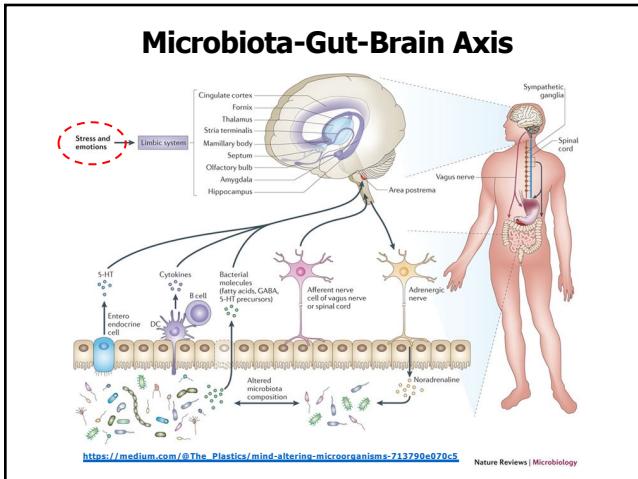
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Microbiota-Gut-Brain Axis

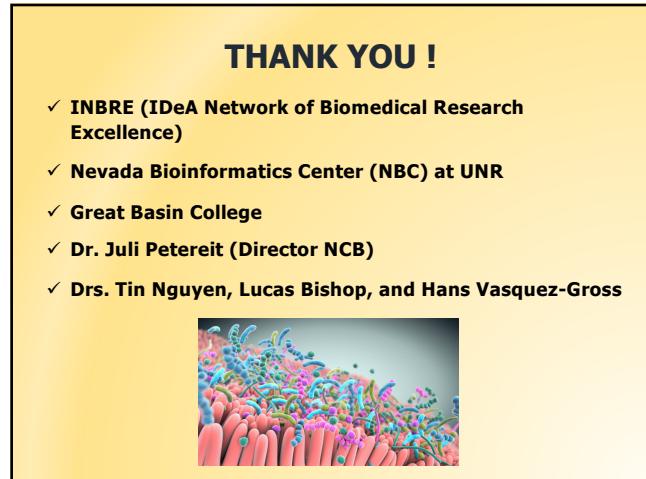
- Two-way communication between the gut microbiota and the central nervous system (CNS) is mediated by several direct and indirect pathways of the gut-brain axis.
- Microbiota affects the expression of genes involved in HPA axis regulation and local metabolism of glucocorticoids in chronic psychosocial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



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