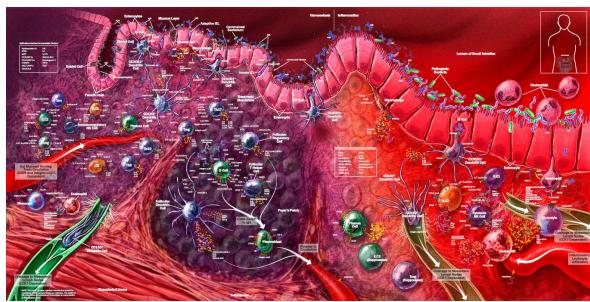


Overview of Gut Immunity and Microbiota

Daniel Bergey, Ph.D. // Great Basin College



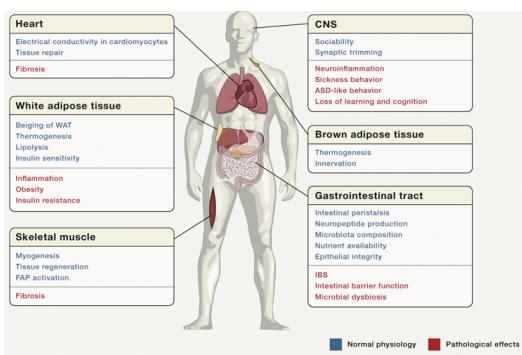
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Discussion Outline

- Immune system overview
- Microanatomy of gut mucosa
- Gut-specific immunity
- Microbiota-immune system link
- Maintenance of gut immune homeostasis
- Inflammation and microbiota
- Intro to microbiota-gut-brain axis

2

Accumulating evidence confirms the immune system's diverse capacity to sense & interact with numerous organ systems (including endothelium), and confirms its central role in maintaining tissue and whole-body homeostasis.



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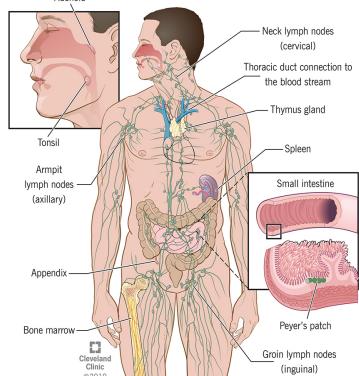
Immune System Overview

Some Terms:

- **Immunology:** study of how we defend against "foreign" (non-self) invaders, such as infectious microorganisms and their toxins, cancer cells, etc.
- **Immunity:** resistance to pathogens and their toxin products.
- **Immune Response:** coordinated/integrated defense response in host to presence of a foreign invader, or its products.
- **Antigen:** a molecule, microbe, etc. that is recognized by immune cells.
- **Immunogen:** a molecule capable of activating an immune response by a host immune system. *All immunogens are also antigens, but not vice versa.*

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Immune System Organs & Tissues



5

Immune System Overview

Classical functions:

Discriminate between self- vs. non/ altered-self, and mediate host defense against invading microbes and tumors.

Emerging functions:

Immune cells also sense complex tissue- and environment-derived signals, including those from the diet and the nervous system.

These responses, in turn, regulate diverse physiological processes throughout the body including nervous system development & function, metabolic state, thermogenesis, and tissue repair.

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Two Integrated Branches of the Immune System

(1) Innate (non-specific; brute bodyguard/killers; no memory):

- First line of host defense; physical barriers and 24-hr sentinels ("first responders"). Phagocytes (eat, digest invaders) → *macrophages, neutrophils, NK cells*
- Quick, but limited, immune response (0-6 hrs).
- Relies on immune cells and mechanisms that are continuously on duty. Hard-wired. No memory.

(2) Adaptive (specific; educated bodyguard/killers; memory):

- Second line of defense (activated if innate overwhelmed); 6-72 hrs
- "Adapts" to specific features of the invader after infection. *Antibodies (specific) produced*
- Mediated by highly specialized, antigen-recognition cells. → *APCs; B and T cells; Killer T cells (CTLS)*.

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Major Immune Cells

Neutrophils: Phagocytic. Short-lived (3-5 days). Circulate in blood & recruited to tissues during infection to destroy invaders (release destructive reactive oxygen intermediates; ROI). 10^{11} cells/day made! ~60% of all circulating WBCs. Innate.

Macrophages: Phagocytic. Epithelium/mucosa, tissues, lymphoid organs. Professional APC (antigen presenting cell). Release cytokines & chemokines that increase blood vessel permeability. Direct migration of circulating neutrophils to infection sites. Innate.

Dendritic cells (DCs): Phagocytic. Reside in tissues, but migrate to lymph nodes when activated. Most potent and versatile APC for activating T-cells and adaptive response. Innate/Adaptive.

Mast cells: Mainly epithelium/mucosa. Recruit eosinophils and basophils, and involved in immune response to parasites and allergic reactions. Innate.

Natural killer cells (NK cells): Lymphocytes generally considered part of the innate immune system because they lack antigen-specific receptors. Circulate in blood to seek-and-destroy "abnormal" cells (e.g., viral-infected cells, tumor cells).

B cells and T cells: Circulate in blood; lymphoid organs. Key cells involved in initiating the adaptive response and antibody production (more detail below).

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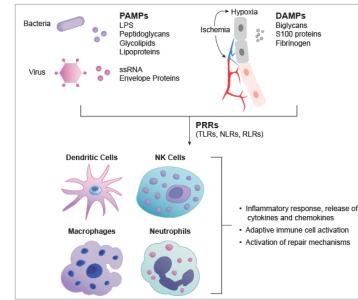
Key Immune Signals and Receptors

- ❑ **Cytokines:** Signal molecules that regulate hematopoiesis and host responses to infection, immune responses, inflammation, and trauma. Some are pro-inflammatory, whereas 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 (patterns) found on various classes of pathogens (e.g., TLR).
- ❑ **PAMPs** (pathogen-associated molecular patterns): Pathogen-derived signal molecules (e.g., lipopolysaccharide, peptidoglycan) recognized by PRRs that elicit immune response. e.g., TLRs
- ❑ **DAMPs** (danger/damage-associated molecular patterns): Host-derived (endogenous) signal molecules that initiate immune responses. *Can also promote pathological inflammatory responses.*

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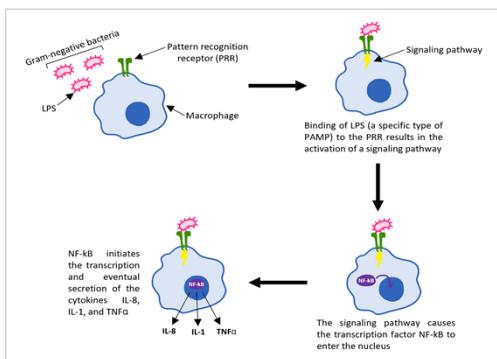
PAMPs & DAMPs interact with PRRs to activate innate immunity & inflammatory responses

- Microbe-derived **PAMPs** drive inflammation in response to infections.
- **DAMPs** are derived from host cells including tumor cells, dead or dying cells, or substances released from cells in response to stress such as hypoxia.



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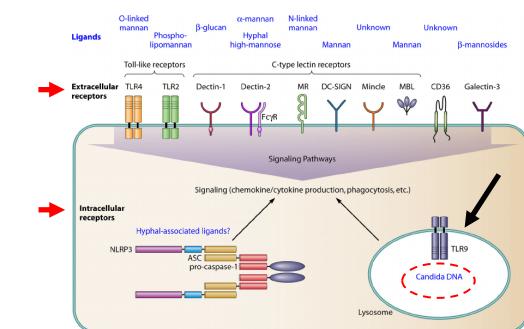
PAMP-PRR Interaction Initiates Immune Cell Activation and Cytokine Release



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Toll-Like Receptors (TLRs) are One Class of PAMPs Found in Vertebrates & Invertebrates

→ E.g., TLR9 recognizes the human commensal fungi *Candida albicans*



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The Adaptive Immune Response Consists of Two Functional Sub-Components

(1) Cell-Mediated Response (killer cells)

- T-cell activation by antigen ("killer" T cells/CTL's)
- Destroy "first response" phagocytes (e.g., macrophages, etc.) that aren't able to eliminate intracellular microbes they've ingested.

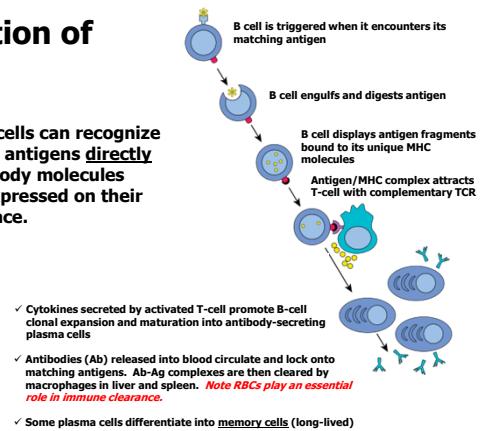
(2) Humoral Response (antibodies):

- B-cell activation by antigen
- Mediated by antibodies (tag invaders for neutralization or destruction).
- Eliminates extra-cellular microbes and their toxins

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Activation of B Cells

- ❑ Naïve B cells can recognize and bind antigens **directly** via antibody molecules (IgM) expressed on their cell surface.

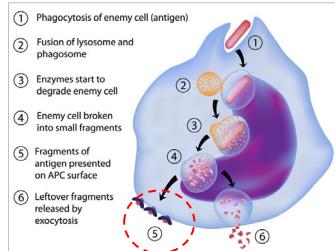


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Professional Antigen Presenting Cells (APCs)

→ Macrophages, Dendritic cells, and B-cells

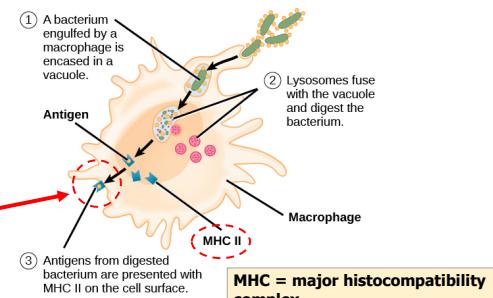
❑ Antigen presentation by APCs



Antigen fragments complexed with MHC molecules displayed on surface. APC effectively "trolling" for a specific T-cell with matching T-cell receptor (TCR).

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Antigen-MHC Complex is Displayed on Surface of APCs



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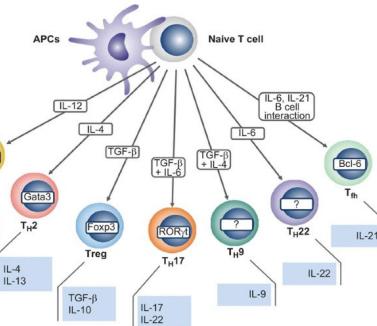
Antigen Presentation and Activation of T-Cells

→ Unlike B-cells, T-cells cannot bind antigen directly

- Antigen/MHC complex displayed on cell surface of APC is recognized by T-cell expressing a specific, matching TCR.
 - Interaction of APC with T-cell interaction activates T-cell initiating adaptive response.
-

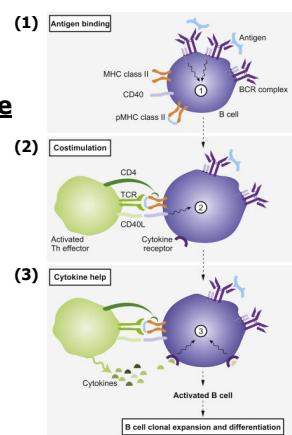
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T-cell Differentiation Pathways are Directed by Existing Immune Context (e.g., antigen type, tissue microenvironment, cytokine profile, etc.)



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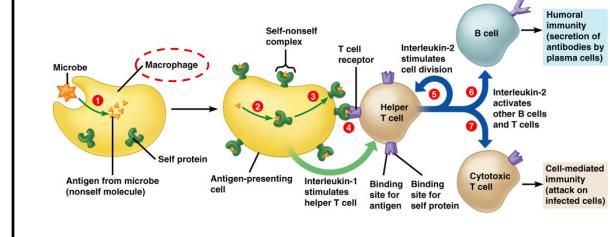
Three Steps are Required for Optimal Activation of Adaptive Immune Response



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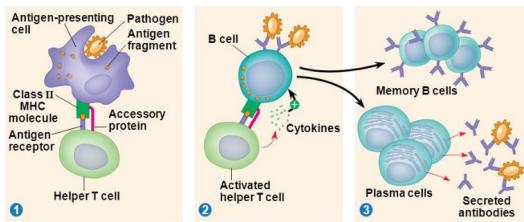
Summary of Adaptive Response Activation and Key Effector Cells

- Recall "professional" APCs = macrophages, dendritic cells, and B-cells)



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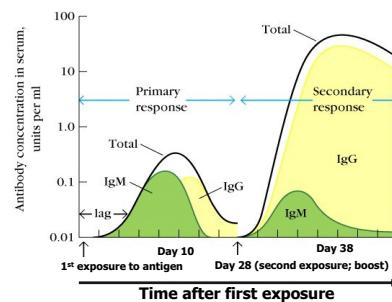
Antibody Production Requires Collaboration between B- and T-Cells in Specialized Lymphoid Tissue (lymph nodes, Peyer's patch, etc.)



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Primary vs. Secondary Antibody Response

- ❑ **Primary response:** IgM predominates initially; low blood titer; low affinity Abs.
- ❑ **Secondary response:** IgG predominates; 10^2 to 10^3 higher blood titer; high affinity Abs



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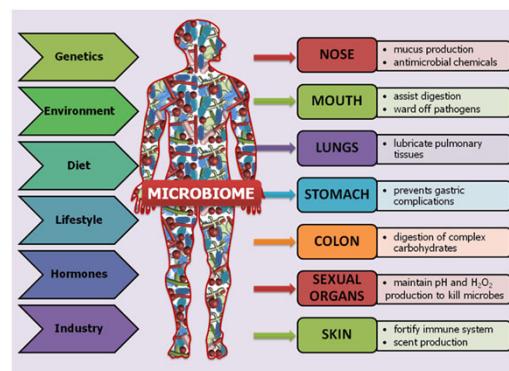
Microbiome: A community of micro-organisms living together in a particular habitat.

- Every human body surface 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 and collections 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, protecting against pathogens – *and neurological development (?)* Altered gut bacterial composition (dysbiosis) is associated with the pathogenesis of many inflammatory diseases and infections.

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

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Microbiomes In 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 am the most important because I control everything".
 - ❑ The heart said: "I am the most important because my beating keeps the body going".
 - ❑ The liver said: "I am the most important since I help in metabolism, excretion, detoxification, etc."
 - ❑ Finally, the GUT said: "No, I am the most important. I digest, absorb, protect, defend, excrete, produce hormones, etc."
 - ❑ **EVERYONE LAUGHED --- 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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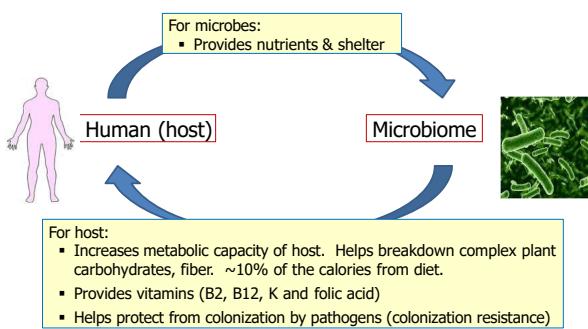
"All disease begins in the gut."

- 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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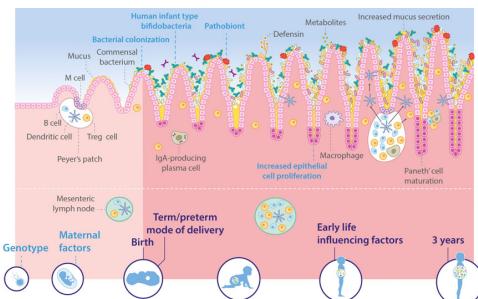
The Gastrointestinal Tract is a Unique Challenge for the Mammalian Immune System

- ❑ The GI tract (GUT) must tolerate the presence of 100 trillion luminal microbes (microbiota), and not respond inappropriately to their products, but still protect the intestinal mucosa from potentially harmful dietary antigens and invading pathogens.
- ❑ The intestinal epithelium, a single layer of cells, is crucial 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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Normal Gut, Microbiota, and Immune Development

- ❑ Early life nutrition is a crucial factor in shaping the gut microbiota, and brain development. Better understanding of the microbiota-gut-brain relationship, and the role of nutrition in early brain and neurological development is a very active area of research.



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Protective Function (barrier effect) of Commensal Microbes

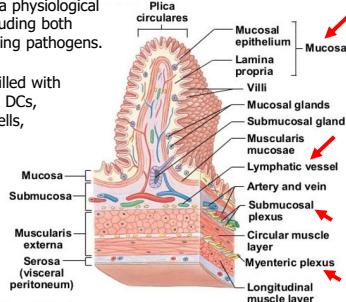
- ❑ Compete and adhere to potential attachment sites on the brush border of intestinal epithelial.
- ❑ Compete for available nutrients.
- ❑ Produce antimicrobial compounds (defensins, bacteriocins).
- ❑ The intestinal mucosa provides a physiological barrier for most microbes, including both commensal bacteria and invading pathogens.

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

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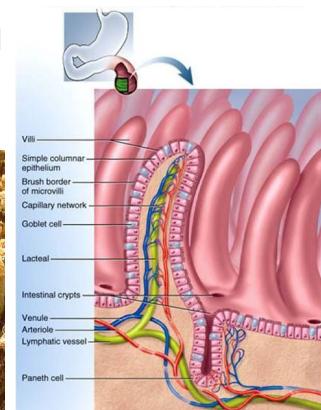
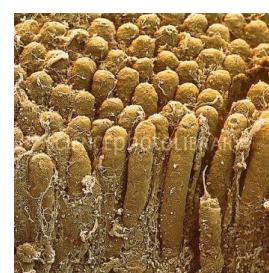
Microanatomy of Gut Wall

- ❑ **Intestinal mucosa** provides a physiological barrier for most microbes, including both commensal bacteria and invading pathogens.
- ❑ The **lamina propria** layer is filled with various immune cells including DCs, Macs, IgA-producing plasma cells, B cells and various T cells.
- ❑ Note extensive innervation of gut ("second brain")



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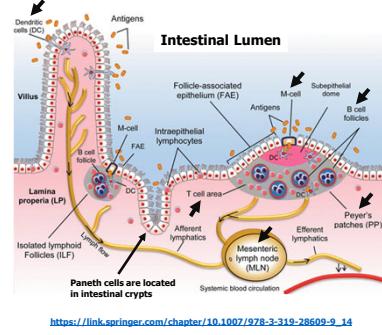
Intestinal Villi and Lymph/Blood Circulation



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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 lymphocytes.
- ❑ GALT is exposed to more antigens than any other part of the body (e.g., commensal bacteria, dietary gut antigens, and invasive pathogens).



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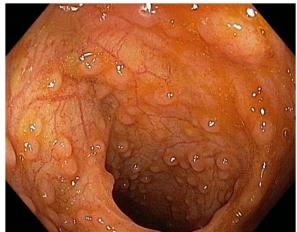
Major Components of GALT

- ❑ **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.

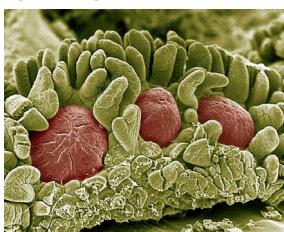
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Peyer's Patches ("Intestinal Tonsils")

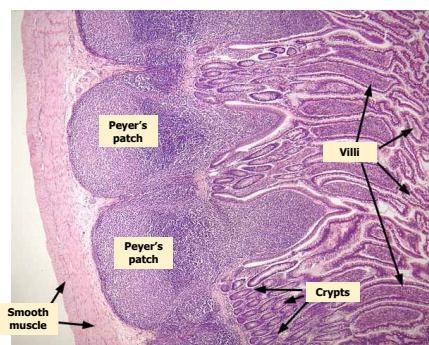
Peyer's patches (PP) in the distal ileum (20-yr old man).



Scanning EM of Peyer's patches (colorized)



Peyer's Patches (Histology)

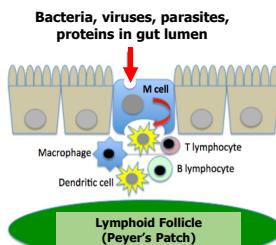


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M Cells play a central role in the mucosal immune response, and immune surveillance

- ❑ M cells (microfold) are embedded in the epithelium, just above Peyer's patches (PP), of mucosal tissues such as intestine, lung, and nasopharynx, and continually transport antigens from lumen into PP.
- ❑ M cells engulf a variety of antigens (bacteria, viruses, parasites, proteins) from the gut lumen, transport them across the cell, and deliver them to immune cells (e.g., DCs) lying just underneath, to activate mucosal immune responses.



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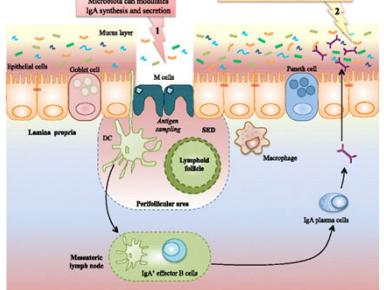
Role of DCs and IgA in Gut Immune Surveillance & Tolerance

DCs continually sample luminal antigens and promote tolerance to microbiota by inducing IgA synthesis in B cells, and promoting the differentiation of Treg cells. Characteristics of intestinal DCs are strongly influenced by the gut microenvironment, including the presence of commensal bacterial metabolites, epithelial cell derived factors, and dietary products.

- ❑ Dendritic cells (DCs) interact with luminal antigens that cross **M cells** of **Peyer's patches (PP)**, and from isolated lymphoid follicles (ILF) that are transported to 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). 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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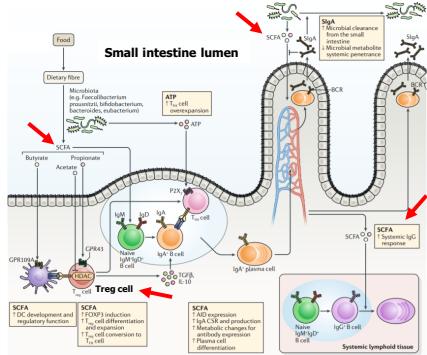
T-regulatory (Treg) Cells and Tolerance to Commensal Microbes

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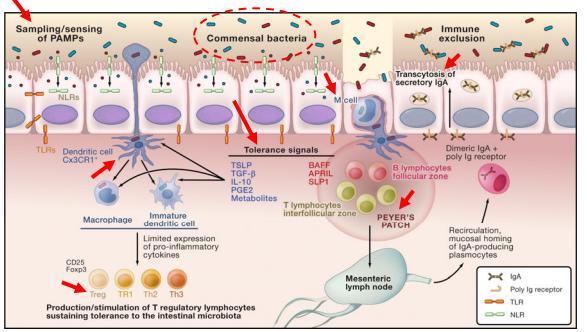
Commensal Gut Microbes Generate Short Chain Fatty Acids (SCFAs) that Induce Treg Cell Differentiation



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

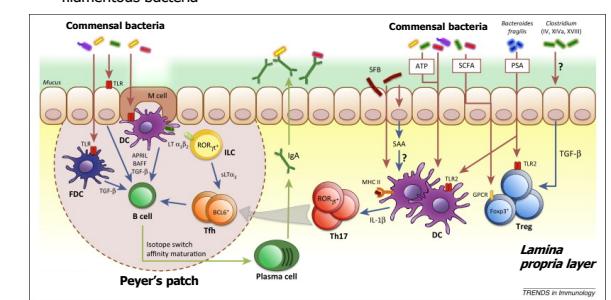
- Potential tissue-damaging, inappropriate T cell responses can be inhibited by immunosuppressive cytokines and regulatory T cells (T reg).



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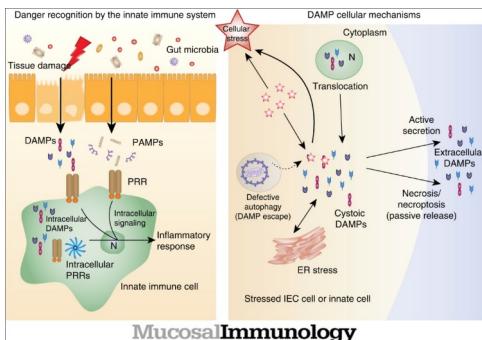
Microbiota and Immune Response in Gut Homeostasis

- 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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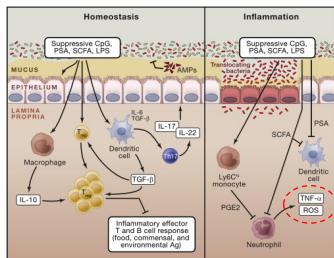
Gut mucosal DAMPs and Inflammation



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Gut Microbiota in Homeostasis and Inflammation

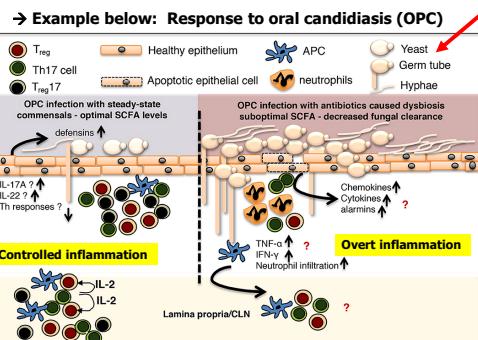
- **Left panel:** Commensal microbes promote induction of **Treg** cells via direct sensing of microbial products or metabolites by T cells or DCs. Other commensal microbes promote the induction of **Th17 cells** that can regulate function and homeostasis of gut epithelial cells.
- **Right panel:** Commensal-derived **metabolites** can have local and systemic effects on inflammatory cells. E.g., **SCFAs** inhibit neutrophil activation. Upon entrance into tissue, inflammatory monocytes can respond to microbial products by producing immune signal molecules (e.g., **PGE₂**) that **limit** neutrophil activation and tissue damage.



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

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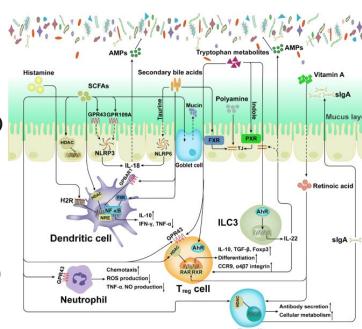
- **SCFAs contribute to immune homeostasis in mucosa, but excessive and suboptimal levels of SCFAs are often associated with inflammation and cancer.**



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Metabolites Derived from Microbiota or Host Mediate Host-Microbiota Interactions

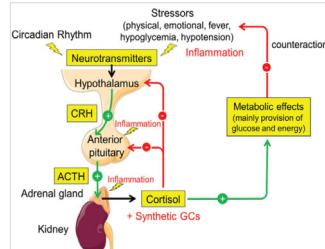
- **SCFAs (short chain fatty acids)**
- **AMPs (antimicrobial peptides)**
- **HADC (histone deacetylase)**
- **TJ (tight junction)**
- **AhR (aryl hydrocarbon receptor)**
- **FXR (farnesoid X receptor)**
- **PXR (pregnane X receptor)**
- **ILC3 group 3 innate lymphoid cell**



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

- HPA axis activation promotes inflammatory responses in both the brain and peripheral tissues. Depressed individuals exhibit elevated levels of cortisol and plasma concentrations of pro-inflammatory cytokines (e.g., CRP).
- HPA axis hyperactivity also promotes development of various cardiometabolic, inflammatory, endocrine, and neural disorders.



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

The Hypothalamic-Pituitary-Adrenal Axis (HPA) and Inflammation (summary)

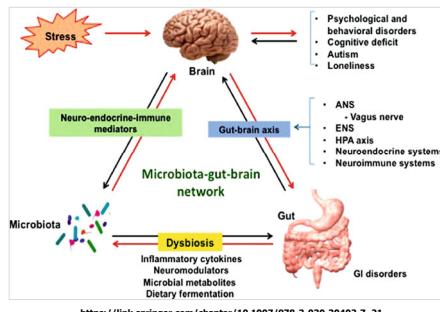
- Activation of the hypothalamic-pituitary-adrenal (HPA) axis and negative feedback regulation. Different stressors (physical, emotional, fever, hypoglycemia, or hypotension) trigger the **hypothalamus** to release **corticotropin-releasing hormone (CRH)**.
- CRH acts on the **anterior pituitary** and induces release of adrenocorticotrophic hormone (**ACTH**), which in turn stimulates the adrenal gland to produce and release **cortisol**.
- Cortisol initiates metabolic effects (e.g., mobilization of glucose and energy), which serve to counteract the stressor. Also directly **suppresses** immune system.
- **Inflammation** can also trigger HPA axis. In the physiological regulation of the HPA axis, cortisol release is terminated by negative feedback regulation of cortisol on the hypothalamus and anterior pituitary. Synthetic glucocorticoids (GCs) applied in GC therapy can cause negative feedback regulation – however, this can lead to adrenal suppression.

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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.

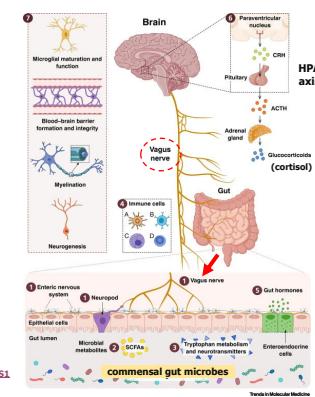


https://link.springer.com/chapter/10.1007/978-3-030-30402-7_21

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Microbiota-Gut-Brain Axis

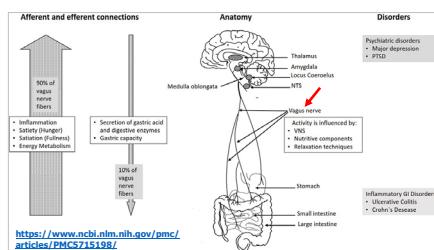
- Recent research demonstrate as an important role for gut microbiota in the regulation of brain development and behavior.
- Host pattern-recognition receptors (**PRRs**) that recognize conserved microbe surface molecules (peptidoglycans, etc.) have emerged as key regulators of gut microbiota-brain interactions.



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Microbiota-Gut-Brain Axis

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



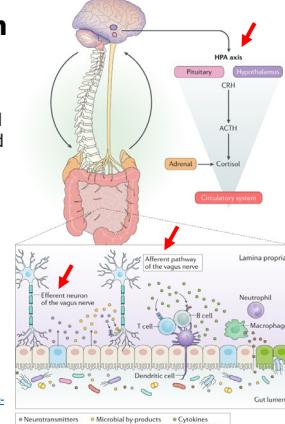
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Microbiota-Gut-Brain Axis

- 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?*)

- <https://www.nature.com/articles/s41579-020-00460-0>
- <https://pubmed.ncbi.nlm.nih.gov/29990567/>
- <https://cen.acs.org/biological-chemistry/microbiome/gut-might-modify-mind/97/114>
- <https://www.nature.com/articles/d41586-021-00260-3>

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