Microbiology

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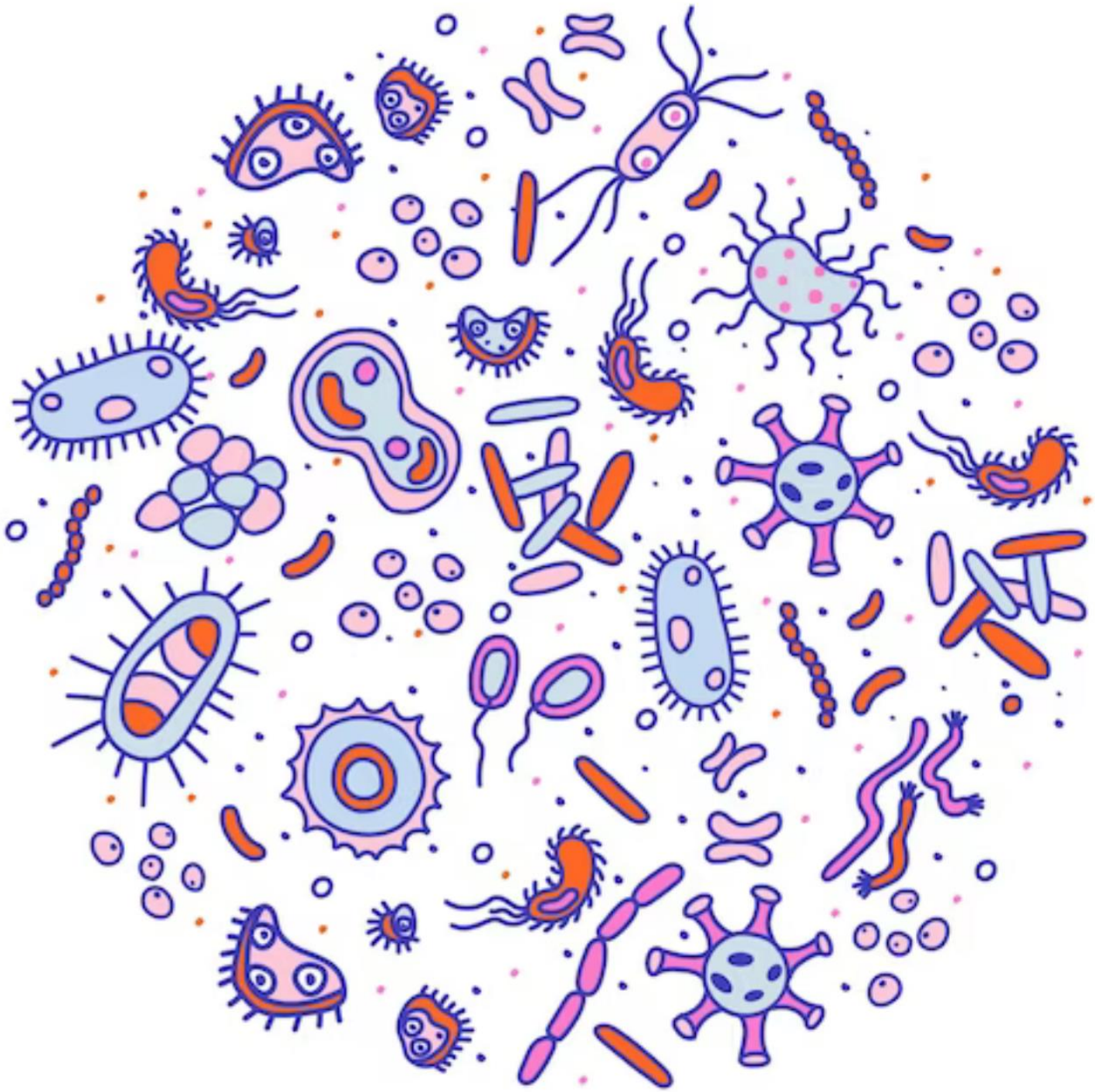

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Natural defense of the gastrointestinal tract (Microbiome & Immune responses)

In this sheet, we will talk about:

1.An overview of the immune responses of GI tract & their distinctive features.
2. Microbiome or commensal organisms (previously called normal flora).

Let's start with key facts regarding the GI tract

Green => slides

Black => doctor's notes

Underlined or red => important

KEY FACTS

The gastrointestinal (GI) tract represents the largest surface area in the body, and requires protection from infectious and non-infectious threats continuously introduced during ingestion.

The presence of microbiome in a healthy large intestine (consisting of at least thousand species of bacteria that live in symbiosis with their host) requires continuous protection during ingestions & preventing any microbial breach caused by the resident microbiome. (Symbiosis is an interaction between two different organisms living in close physical association)

Diarrheal disease caused by enteric pathogens remains a leading cause of childhood mortality. It is both preventable and treatable.

The mucosal immune system of the gut is faced with the extraordinary challenge of coexisting with the microbiome and simultaneously preventing a breach in a single layer of epithelial cells.

NATURAL DEFENSE

1.Anatomical & physiological barrier : skin (epithelium, where cells joined by tight junctions and mucous membrane), oral mucosa , intestinal epithelium.

2.chemical barriers such as: The acidity of the stomach (Unfavorable for most microbes)**, complement and antimicrobial proteins:**

antibacterial enzymes: lysozymes, secretory phospholipase A2 (Paneth cells). (Innate)

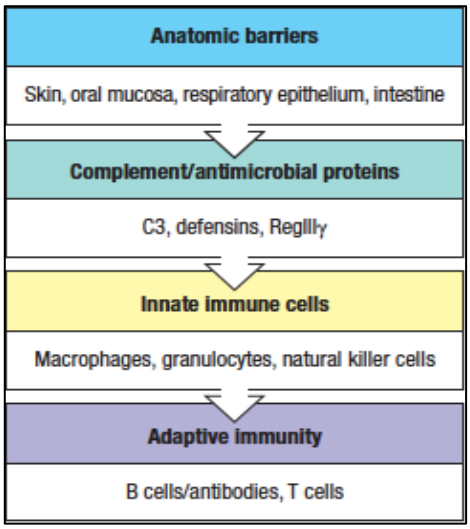
antimicrobial peptides: defensins, cathelicidins, and histatins. (Innate)

Saliva contains numerous hydrolytic enzymes (secretory phospholipase A2). (Innate)

Antibody production and secretion of Secretory IgA. Which is the predominant class of antibodies in mucosal immune system, it is produced locally by plasma cells.

-Chemical barriers include activation of innate immune system namely: the complement system, antimicrobial proteins (enzymes, peptides), and secretory molecules such as Phospholipase A2 & mucus.

PROTECTION AGAINST PATHOGENS RELIES ON SEVERAL LEVELS OF DEFENSE



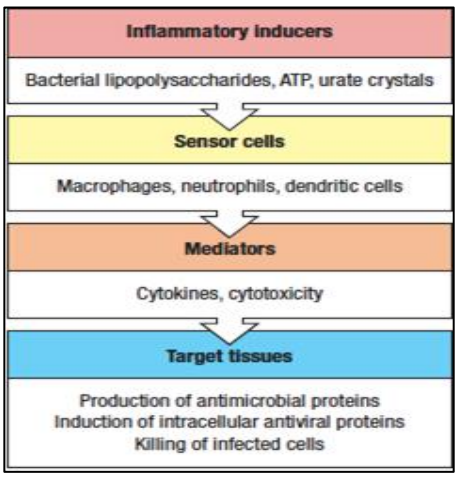
1. 1st line of defense against the invasion by potential pathogens and commensal microorganisms is the thin layer of epithelium that covers all these surfaces. However, the epithelium can be breached relatively easily so it needs to be supplemented by cells & molecules of mucosal immune system.

2. second line are various chemical and enzymatic systems including complement system & antimicrobial proteins. They act as an immediate antimicrobial barrier near the epithelium.

3. If epithelium is breached, nearby various innate lymphoid cells coordinate a rapid cell-mediated defense.

4. And if the pathogen overcomes the previous barriers, the adaptive immunity takes a role (slower).

THE IMMUNE SYSTEM IS ACTIVATED BY INFLAMMATORY INDUCERS THAT INDICATE THE PRESENCE OF PATHOGENS OR TISSUE DAMAGE



Cell-mediated immunity proceeds in a series of steps:

1. Inflammatory inducers:

A. PAMPs (Pathogen Associated Molecular Patterns) → indicate presence of an invading microbe.

B. DAMPS (Damage Associated Molecular Patterns) → indicate presence of cell/tissue damage.

2. Sensor cells: detect the inflammatory inducers by expressing various innate receptors called pattern

recognition receptors (PRRs) → they produce a variety of mediators that act directly in defense or further propagate the immune response.

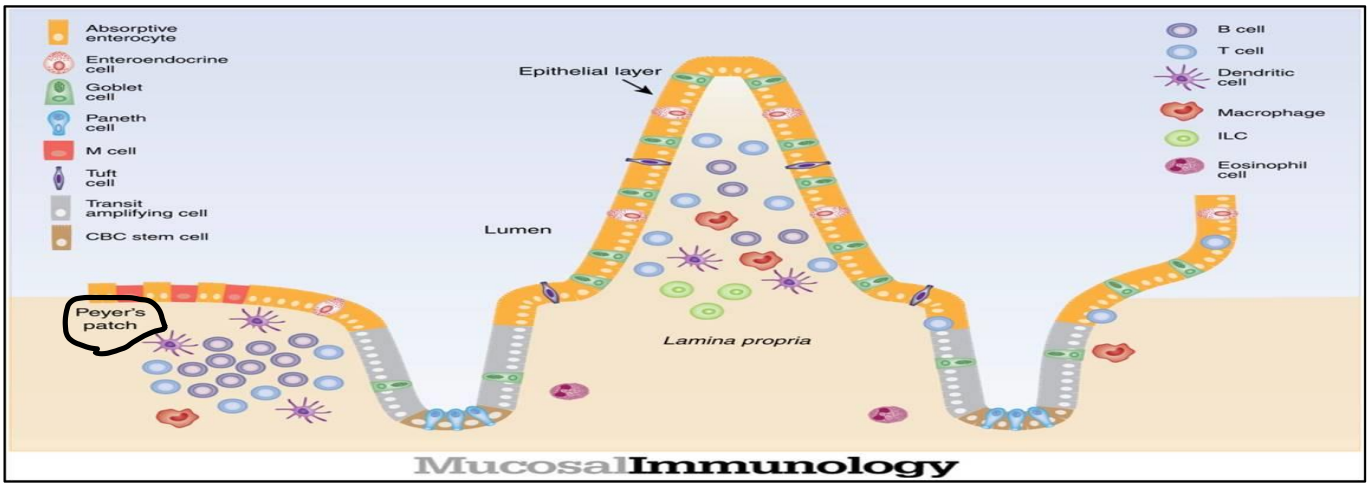
3. Mediators (Cytokines): act on various tissues such as epithelial cells (to induce antimicrobial proteins and peptides), and they resist intracellular viral growth. They also can act on other immune cells such as the Innate lymphoid cells (which produce other cytokines) to amplify the immune response.

EPITHELIAL SURFACES OF THE BODY PROVIDE THE FIRST BARRIER AGAINST INFECTION

Most of the enzymatic breakdown of food occurs in the small intestine where the surface area available for nutrient absorption is maximized by finger-like protrusions called villi, which are predominantly covered by absorptive columnar epithelial cells known as enterocytes (Intestinal epithelial cells IECs).

Between villi are the crypts of Lieberkuhn, invaginations that shield stem cells, which give rise to all the IEC lineages.

These crypts include stem cells, and mucus-producing goblet cells found throughout the GI tract, and Paneth cells located in the base of the small intestinal crypts where they secrete antimicrobial molecules.



THE INTESTINAL EPITHELIUM IS A UNIQUE COMPARTMENT OF THE IMMUNE SYSTEM

The forest of villi is interrupted by occasional lymphoid nodules referred to as Peyer's patches. In the left side of the previous figure

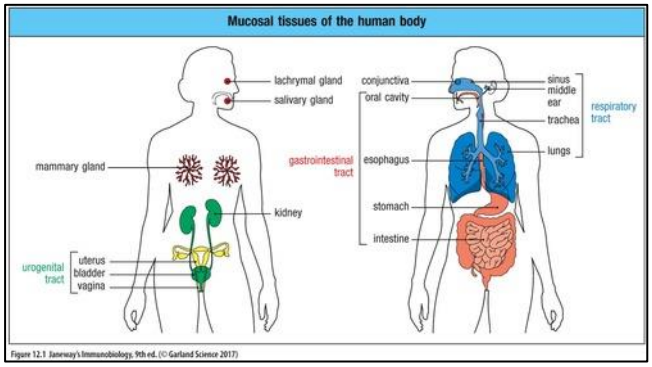
The epithelium above Peyer's patches include microfold (M) cells, which are specialized IECs that allow luminal contents to pass through and encounter antigen presenting cells (APCs) below.

M cells increase vulnerability to infection by serving as a point of entry for pathogens eg. *Salmonella enterica , Shigella Yersinia pestis.* Pathogens can take advantage of M cells to infect the body.

Abundant intraepithelial lymphocytes (IELs) such as macrophages and dendritic cells present as organized tissue and scattered (disorganized) through the mucosa of the intestine and the underlying layer of connective tissue (called lamina propria), **More than 90% of the IELs in the small intestine are T cells, and around 80% of these carry CD8, in complete contrast to the lymphocytes in the lamina propria** (predominantly B-Cells).

MUCOSAL TISSUES OF THE HUMAN BODY

The mucosal immune system differs from the systemic immune system because most internal tissues remain largely free from active microbial growth. In contrast, the skin and mucosal lining organs, which directly interface with the external environment, continuously encounter environmental microbes. These surfaces serve as the battleground where most pathogens invade the mucosal immune system, particularly in the gut.



The gut mucosal immune system likely evolved as the vertebrate immune system's earliest defense mechanism, driven by the necessity to manage the vast population of commensal bacteria that co-evolved alongside vertebrates.

Mucosal surfaces have specialized immune structures that orchestrate responses to environmental microbial encounters.

An enormous area to be protected!

The mucosal immune system comprises the internal body surfaces that are lined by a mucus-secreting epithelium, including:

1.the gastrointestinal tract.

2.the upper and lower respiratory tract.

3.the urogenital tract, and the middle ear.

4.the exocrine glands associated with these organs, such as the conjunctivae and lacrymal glands of the eye, the salivary glands, and the lactating breast.

DISTINCTIVE FEATURES OF THE MUCOSAL IMMUNE SYSTEM

-In contrast to the systemic immune system, the mucosal immune system is bigger and encounters a wider range of antigens (and much more frequently than the systemic immune system). Thus, the mucosal immune system has special anatomical features and specialized mechanisms for the uptake and the unusual effector and regulatory response that prevents unwanted immune reaction to food.

Distinctive features of the mucosal immune system	
Anatomical features	Intimate interactions between mucosal epithelia and lymphoid tissues
	Discrete compartments of diffuse lymphoid tissue and more organized structures such as Peyer's patches, isolated lymphoid follicles, and tonsils
	Specialized antigen-uptake mechanisms, e.g., M cells in Peyer's patches, adenoids, and tonsils
Effector mechanisms	Activated/memory T cells predominate even in the absence of infection
	Multiple activated 'natural' effector/regulatory T cells present
	Secretory IgA antibodies
	Presence of distinctive microbiota
Immunoregulatory environment	Active downregulation of immune responses (e.g., to food and other innocuous antigens) predominates
	Inhibitory macrophages and tolerance-inducing dendritic cells

-We will cover some of these distinctive features in the next slides

MUCOSA-ASSOCIATED LYMPHOID TISSUES (MALT)

-In GI tract it's called Gut-Associated Lymphoid Tissue (GALT)

Collectively, the mucosal immune system is estimated to contain as many lymphocytes as all the rest of the body, and they form a specialized set of cells obeying somewhat different rules of recirculation from those in the other peripheral lymphoid organs.

The gut-associated lymphoid tissues (GALT) include the Tonsils, adenoids, appendix, (large aggregates of lymphoid tissues covered by squamous epithelium and jointly form the Waldeyer's ring at the back of the mouth, around the entrance of the gut and airway), **and specialized structures in the small intestine called Peyer's patches** (only found in small intestine)**, Isolated lymphoid follicles -they collect antigen from the epithelial surfaces of the gastrointestinal tract are found throughout the intestine.**

In Peyer's patches, which are the most important and highly organized of these tissues, the antigen is collected by specialized epithelial cells called microfold or M cells.

GUT ASSOCIATED LYMPHOID TISSUE (GALT)

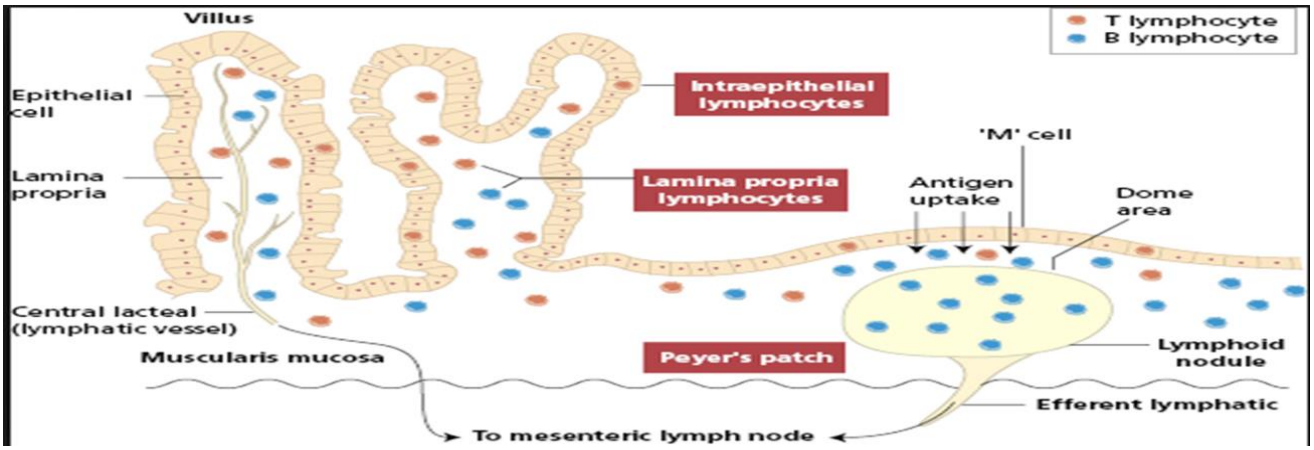
GALTs lie in the intestinal wall itself. Separated from the content of intestinal lumen by a single epithelium layer.

Mesenteric lymph nodes and Coda lymph nodes (drains from the gut, they are the largest lymph nodes in the body) are connected to Peyer's patches by efferent lymphatic vessels. Together, they are the sites for T, B cells antigen presentation, and are also responsible for the induction phase of the immune response.

Peyer's patches are important sites for initiation of immune response in the gut, visible to the naked eye (distinctive dome-like aggregates of lymphoid cells that project into the intestinal lumen).

Peyer's patches and Mesenteric lymph nodes contain discrete **T cell areas**.

The main lymphocytes in the isolated follicles (lymphoid nodules) is **B cells**.



MUCUS FORMS A KEY PROTECTIVE BARRIER IN THE GUT

The intestinal mucus protects the epithelium from bacteria by promoting their clearance & separating them from epithelial cells (like a buffer area) thereby inhibiting inflammation & infection. This explains how we can harbor such a large number of bacteria in our gut, especially in colon.

In turn, microorganisms have developed mechanisms for overcoming this well-organized mucous protective system.

Goblet cells secrete a heavily glycosylated mucins that oligomerize through disulfide bonds to form mucus. O-linked oligosaccharide modification of the conserved Proline-Threonine-Serine (PTS) repeats in the mucin domain maintains the integrity of the epithelial barrier. Mucin Type II is the major component of mucous.

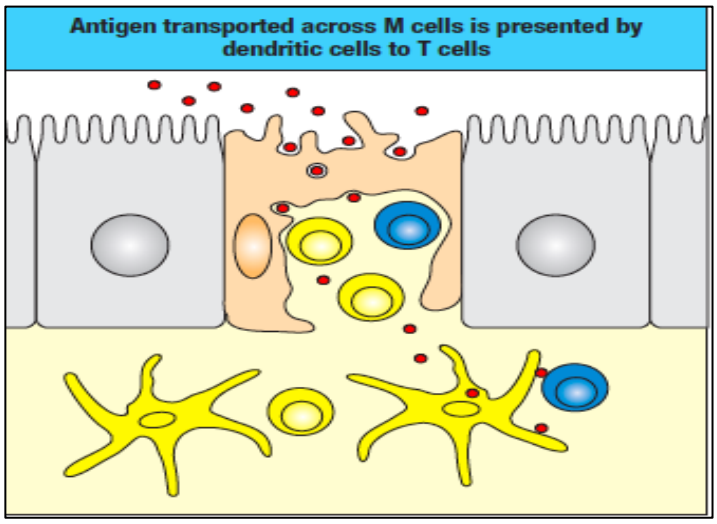
These glycan chains create sticky binding sites in mucus that trap microbes along with antibodies, antimicrobial molecules, and even bacteriophages that can kill the ensnared bacteria.

A formidable barrier to invasion, by trapping microbes and other particles. At the same time, it acts as a scaffolding to retain IgA antibodies and antimicrobial peptides that have been secreted into the lumen across the epithelium.

Mucus is also slippery in nature, meaning that trapped materials can then be expelled easily by normal peristaltic movements.

Mucous has another crucial role which is trapping & holding up IgA antibodies (the predominant antibody class in GI tract).

UPTAKE AND TRANSPORT OF ANTIGEN BY M CELLS



Antigens present on the mucosal surfaces must be transported from the lumen across epithelial layer to be presented to APCs in the extracellular space before stimulating the mucosal immune system.

The previous figure illustrates the process of antigen passage through the M cells (microfold cells). These M cells possess a convoluted basal membrane, facilitating close interaction with lymphocytes and other immune cells. This feature promotes the local transport of antigens absorbed from the intestine by the M cells, delivering them to dendritic cells and other immune cells for antigen presentation.

The intestine has distinctive routes and mechanisms of antigen uptake.

For several bacteria this may involve specific recognition of the bacterial FimH protein found in type 1 pili by a glycoprotein (GP2) on the M cell. This material is transported through the interior of the cell in membrane-bound vesicles to the basal cell membrane, where it is released into the extracellular space—a process known as transcytosis.

Because M cells lack a glycocalyx and so are much more accessible than enterocytes

TRANSCYTOSIS OF SECRETORY IGA

Here we have the immunoglobulin that is secreted from the gut and needs to reach the lumen (predominant class of antibody in the GIT is IgA).

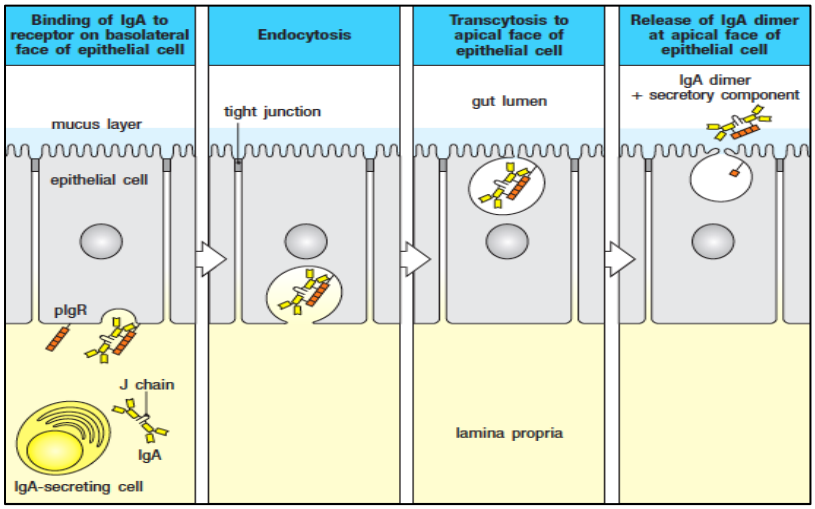
Nature of IgA depends on its location:

Blood → monomer

Mucosa →usually a dimer (2 monomers linked by J chain).

To reach its target, IgA must be transported across epithelium by polymeric immunoglobulin receptor in a vesicle.

The bound complex undergoes transcytosis by a vesicle to reach the apical surface where the polymeric Ig receptor is cleaved leaving the extracellular IgA binding component bound to IgA molecule. Additionally, a



part of the polymeric immunoglobulin receptor remains associated with the IgA (known as the secretory component).

The resulting antibody is protected from proteolytic cleavage and is referred to as secretory IgA (now it can bind to and neutralize pathogens and toxins in the gut lumen).

WHAT IS THE MICROBIOME?

Not all microbes are pathogens. Many tissues, especially the skin, oral mucosa, conjunctiva, and gastrointestinal tract, are constantly colonized by microbial communities —called the microbiome; used to be called "normal flora".

or Commensals (since they can form a symbiosis relationship with the host), the normal flora denotes the microorganisms that inhabit the skin, mucous, etc.... of the healthy person.

All the microbes (microbiota) in and on our bodies Includes bacteria, viruses, and eukaryotes.

Also including archaea, fungi that cause no damage to humans.

symbiotic relationship with the human host- mutualism.

Vast numbers on body sites are usually exposed to environment and not usually inside tissue One-gram faeces contains > world's population.

10 trillion human cells, 100 trillion bacteria –Human body

The ratio is 10:1. (most of them are in the guts especially large intestine, most are confined within the intestinal lumen by a protective layer of the mucous membrane (Mucous membrane plays a buffering role in which separates the microbiome from a direct contact with the epithelium), and they don't cause damage because it can't penetrate mucosa and reach epithelium, unlike pathogenic bacteria which can penetrate the epithelial cells and spread into the underlying tissue.

>100x more genetic material in microbes than human genome! Ironically, we are more microbes than humans!!

Many thousand species (yet only about 100 are pathogens).

The majority of microbiota live in our gut, the distal you go the larger population you will find.

WHERE ARE THEY?

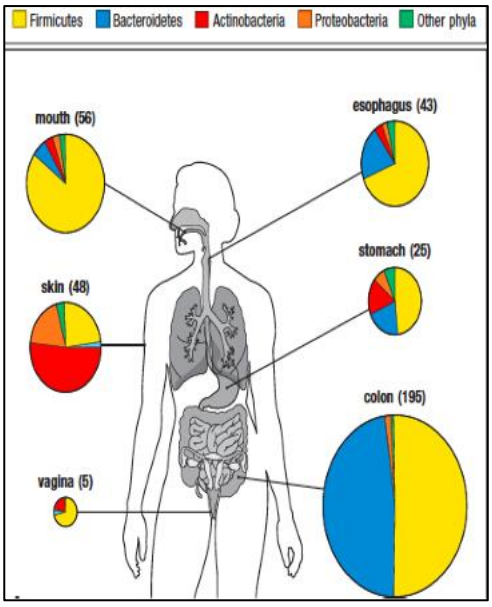
These pie charts show different species at different sites. The 4 bacteria mentioned in the image are the majority of commensal bacteria.

1.Firmicutes: like lactobacilli and the clostridium species.

2.Actinobacteria: like bifidobacterium, the most common inhabitant bacteria in newborn babies.

3.Bacteroidetes: Bacteroidetes fragilis, which are anaerobes, are among the common commensals of the colon.

4.Proteobacteria: E. coli or proteus are the major commensal bacteria.



• On body surface, not inside (blood, deep sterile tissue).

• Mainly in gastrointestinal tract. Flora is different at different sites.

• Depends on environment.

• Acid, bile, and pancreatic secretions hinder the colonization of the stomach and proximal small intestine by most bacteria.

• However, bacterial density increases in the distal small intestine, and in the large intestine rises to an estimated 1011–1012.

WHO ARE THEY?

• Two main phyla in intestine: Firmicutes and Bacteroidetes.

• At phyla level composition is similar between humans and mice.

• Much individual variation, many species.

Different Microbial identities based on the place the surveyed sample was taken from.

• Shared by humans, thought to be core microbiome of 130 species, plus many others.

We classify skin and mucous membrane microorganisms to:

1.Resident (fixed types of microorganisms, found at a given area, and at a given age, and they re-establish themselves when distributed).

2.Transient (Nonpathogenic, but potentially pathogenic. Inhabits the skin or mucous membrane for hours, days, or weeks).

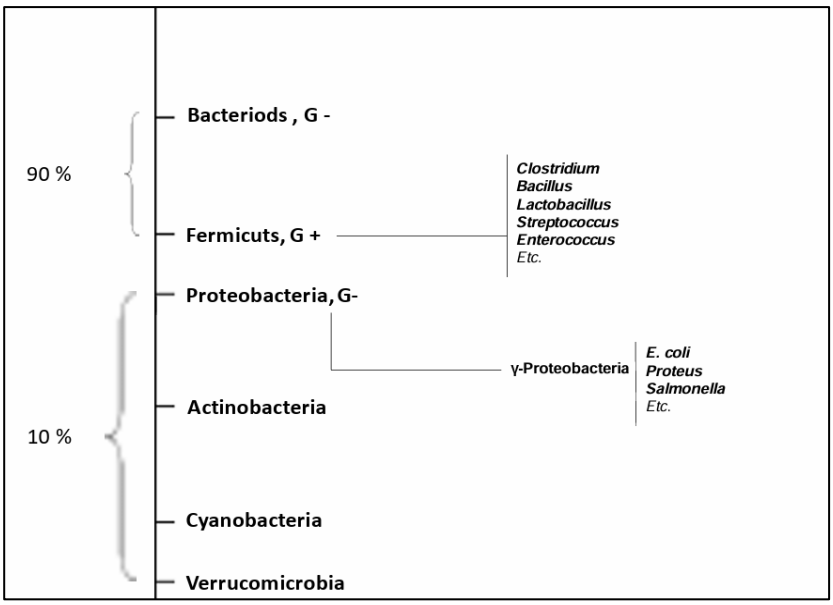
EUBACTERIA

This image illustrates the various types of commensal bacteria that inhabit the human host. Notably, these include Firmicutes and Bacteroides, which are the most common commensal bacteria in the gastrointestinal tract, constituting over 90% of our microbiome.

Additionally, we find Proteobacteria, represented by species like E. coli, Proteus, and Salmonella.

Furthermore, Actinobacteria play a crucial role, with the representative species in this class being Bifidobacterium.

As previously discussed, the microbial identity varies across samples taken from different body sites.



METHODS TO STUDY BACTERIAL MICROBIOTA (NOT EXPLAINED IN THE RECORDED LECTURE)

- **Selective plating –strictly anaerobes, need community to grow!**
- **Besides selective plating, we can stain DNA to get total numbers and idea of population.**
- **PCR to amplify 16S RNA.**
- **Fluorescently tagged DNA oligonucleotides to label 16sRNA sequences.**
- **Microbiota from feces of mouse stained with cybergreen DNA stain.**

WHAT INFLUENCE MICROBIOTA ?

- **Environment: who you first contact (way of birth!), Temp and humidity.**

Natural vaginal birth→ baby will have lactobacilli and Bifidobacterium species of mother.

Caesarean section→ skin microbiota of mother like S. aureus.

- **Nutrition: meat, vegetables.**
- **Hormones: estrogen, insulin.**
- **Genetic constitution: receptors on mucosal surfaces.**
- **Antibiotics: eliminate some which permit others to thrive.**
- **Foreign objects: valves, catheters.**

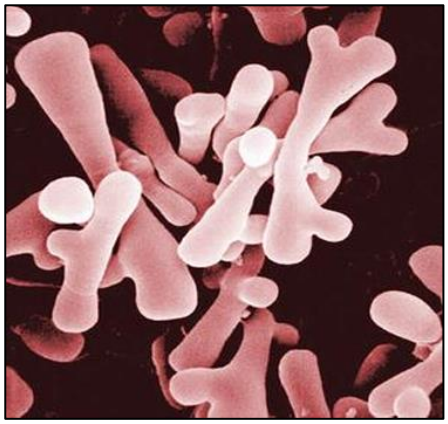
COLONIZATION IS IMMEDIATE AND FOR LIFE

At birth, the intestines are sterile, but soon the organisms are introduced from food, mother's vaginal canal or exposure to fecal or skin microbiota. Those are major factors in determining early microbial profile.

- **Acquired at birth, from environment.**
- **Ingestion of food, fluids, inhalation.**
- **Microbiota established rapidly.**
- **Mature & stabilizes later.**

FOOD CONSUMPTION INFLUENCE MICROBIOTA OF THE SMALL INTESTINE

- **Bifidobacterium Spp. Are the primary faeces inhabitants shortly after birth as child shifts from mother's milk to solid food the microbiota shifts to a more mixed population – other anaerobic bacteria Cl. difficil spp.**



Bifidobacterium are the most common bacteria in infants.

- **Bifidobacterium are anaerobic, Gram + branched rod-shaped bacteria**

WHAT DO THE MICROBIOTA DO FOR US?

The important functions of the microbiota can be divided into three major categories:

1.**Microbial antagonism –space and nutrients competition! Plugs up sites, consumes nutrients, produces inhibitory substances, affects pH and oxygen.**

protective function: in which the resident bacteria displace and inhabit potential pathogens indirectly by competing for nutrients and receptors or directly through the production of antimicrobial factors such as: bactericidin and lactic acid. So, they either fight or compete for space and nutrients on mucous membranes and the skin. the resident microbiota may prevent colonization by pathogens and possible disease through a process known as **bacterial interference**.

2.Nutritional benefits Vitamin K, B12, Steroid metabolism (breaks down bile acids) Organic acid production, Food breakdown.

Some commensal organisms perform important functions as in the case of the bacteria that aid cellulose digestion in the stomach of dominance the difference between commensal organism and pathogens lie as we mentioned before and whether they induce damage to these cells.

3.Stimulate and enhance host defenses, need normal flora to develop normal immune system.

commensal organisms are important for the development and function of the mucosal immune system.

WHAT ARE THE HARMFUL EFFECTS OF MICROBIOTA?

• Pathogenic potential

1. If introduced into other sterile body sites – Urinary tract infections, septic shock, trauma, etc.

2. If host status changes (immunocompromised, nosocomial).

• Gaseous byproducts, Fermentation byproducts: Hydrogen disulfide, methane 300 ml/day in gas produced.

➢Perturbations in the balance between the various species of bacteria present in the microbiota (dysbiosis) have been found to increase susceptibility to a variety of disease.

An example of dysbiosis: the imbalance of microbiota after antibiotics abuse. As in pseudomembranous colitis.

• Changes in lifestyle.

The growing recognition is that microbiota can influence our mental health, overall well-being, and susceptibility to a wide range of diseases. These include cancer, cardiovascular conditions, metabolic disorders, obesity, allergies, autoimmune diseases, as well as autism and other mental health disorders.

HYGIENE THEORY!

Or microbiome depletion theory or microbial diversity hypothesis, or the lost friend theory. It's a national explanation between microbial exposure and inflammatory disorders.

• Do we live too cleanly in childhood (developed countries only)?

• Last 50 years of infectious diseases: all the major diseases have plummeted (rheumatic fever, hepatitis A, tuberculosis, mumps, measles).

• For other diseases mainly immune mediated there is a profound increase (Crohn's disease, multiple sclerosis, type-1 diabetes, asthma).

Microbiome depletion theory: lack of exposure to pathogens in childhood increases susceptibility to allergic diseases by suppressing natural development of immune system and defects in immune tolerance.

MICROBIOTA AND DISEASE

1.Obesity

o Increased proportion of Firmicutes .

o Related to ability of microbiota to harness energy from food.

(obese people have lower diversity of microbiota with increased proportion of enzymes so they are more efficient at digesting food and harvesting calories).

2.Inflammatory Bowel Diseases IBD

o Microbial community imbalances

o increased Proteobacterium, depleted Firmicutes and Bacteroidetes

3.Type I Diabetes

o Interaction of intestinal microbes with innate immune system

4.GI Cancers

o H. pylori (the only colonizer of stomach).

5.Association of various species with colorectal cancer

6.Oral diseases

o Cavities and gingivitis disease Most common infectious disease worldwide

7.Allergy-like (atopic) diseases

-Eczema, allergies, asthma

-Hygiene hypothesis Induction of tolerance (early exposure)

-Antibiotic treatment, C section increase rates of asthma

8.Pseudomembranous colitis

-Follows antibiotic treatment (which alters gut microbiota)

-Caused by Clostridium difficile

-Fecal transplants shown to improve outcome

-**The most Common cause of diarrhea after antibiotic use.**

-Treated by oral vancomycin.

MICROBIOTA AND THE IMMUNE SYSTEM (NOT EXPLAINED IN RECORED LECTUE)

1. Recently realized microbiota plays a key role in immune system development.

2. Germ free (microbiota free) animals have poorly developed immune system.

3. Activation of Toll-like receptors (TLRs) needed for development.

4. Segmented Filamentous Bacteria (SFB) are needed for Th17 cells.

-Critical T cell lineage.

-Germ free mice lack Th17 cells.

-Antibiotics affect Th17 levels.

5. Very recently shown that Treg cells affected by microbiota.

TO MANIPULATE MICROBIOTA 1

1.Probiotics

Live bacteria such as Lactobacilli consumed orally Some protective benefits.

-probiotics **are live microorganisms** intended to provide health benefits when consumed, generally by improving or restoring the gut flora. probiotics are considered generally safe to consume but may cause bacterial host interaction and unwanted side effects in rare cases.

2.Prebiotics

Sugars and other foodstuffs are used to alter microbiota.

On the other hand, prebiotics are **compounds** in food that induce the growth or activity of beneficial microorganisms such as bacteria or fungi.

3.Immunomodulators

Inflammation affects microbiota. (steroids, immunosuppressants).

Drugs that adjust the immune system such as those used in cancer treatment.

4.Antibiotics

Would increase resistance.

5.Phage therapy

Target specific population (resistance rapidly)

bacteriophage therapy has been used to modulate the microbiota. However, it is noticed that the resistance develops rapidly.

6.Fecal transplants

Used in C. difficile infections.

May need to deplete current microbiota.

It's a clinical procedure that restores healthy bacteria in the colon by introducing a stool from a healthy donor through colonoscopy.

7.Use microbiota products

A bacterial polysaccharide from Bacteroides fragilis affects T cell population and Th1/Th2 balance

8.Need other methods!