GI

MICROBIOLOGY

 # 1

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NATURAL DEFENSE OF THE GASTROINTESTINAL TRACT (MICROBIOME & IMMUNE RESPONSES)

❖ Key facts:

1- The gastrointestinal (GI) tract represents the largest surface area in the body (400 square meters, that is 200 times more than the skin), and requires protection from infectious and non-infectious threats continuously introduced during ingestion.

2- The mucosal immune system of the gut is faced with the extraordinary challenge of coexisting with microbioms and simultaneously preventing a breach in a single layer of epithelial cells. (healthy large intestines are colonized by thousands of bacterial species that live in symbiosis with the host, they are called commensal microorganisms or microbiome)

GIT is the most tract exposed to pathogens (ingesting food and drinking) so it needs continuous protection from both these pathogens and imbalances caused by microbiome.

3- Diarrheal disease caused by enteric pathogens, it's the most common symptom of GIT infection, and remains a leading cause of morbidity and mortality worldwide especially in children. It is both preventable and treatable.

❖ Natural defense:

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

2- Chemical barriers such as The acidity of the stomach (unfavorable for most microorganisms)**, complement and antimicrobial proteins (innate immunity).**

a) **antibacterial enzymes: lysozymes, secretory phospholipase A2 (Paneth cells). (innate)**

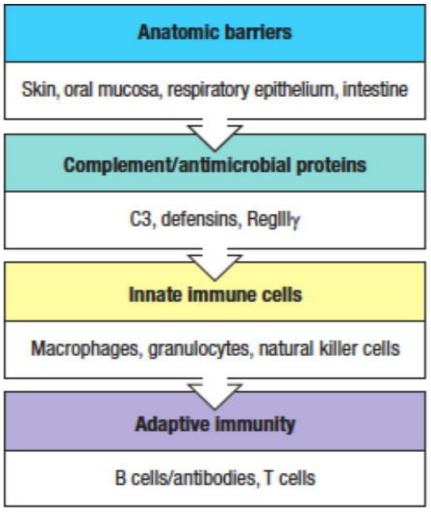
b) **antimicrobial peptides: defensins, cathelicidins, and histatins. (innate)**

c) **Saliva contains numerous hydrolytic enzymes (secretory phospholipase A2). (innate)**

d) **Antibody production and secretion of Sec. IgA** (by plasma cells of mucosal wall).

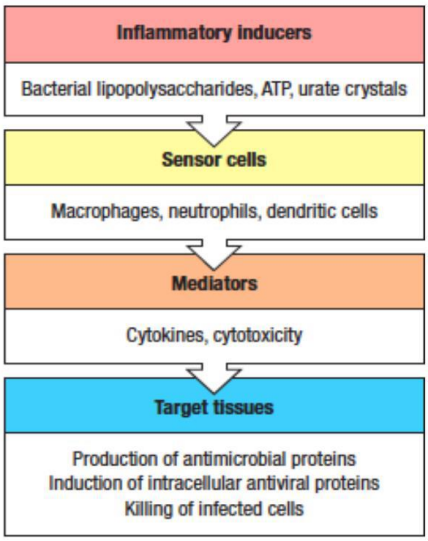
❖ Protection against pathogens relies on several levels of defense:

1st line of defense against the invasion by potential pathogens and commensal microorganisms is the thin layer of epithelium that covers all these surfaces, but it is easily breached so it needs to be supplemented by mucosal immune cells and molecules Second line of defense has various chemical and enzyme systems including complement that act as immediate antimicrobial barrier near the epithelium (if the pathogen overcomes then adaptive immunity takes role)



❖ The immune system is activated by inflammatory inducers that indicate the presence of pathogens or tissue damage:

• **Inflammatory inducers:**
a- PAMPS (pathogen associated molecular patterns) → indicate presence of invading microbe
b- DAMPs (damage associated molecular patterns) → indicate presence of cell/tissue damage.



• **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.

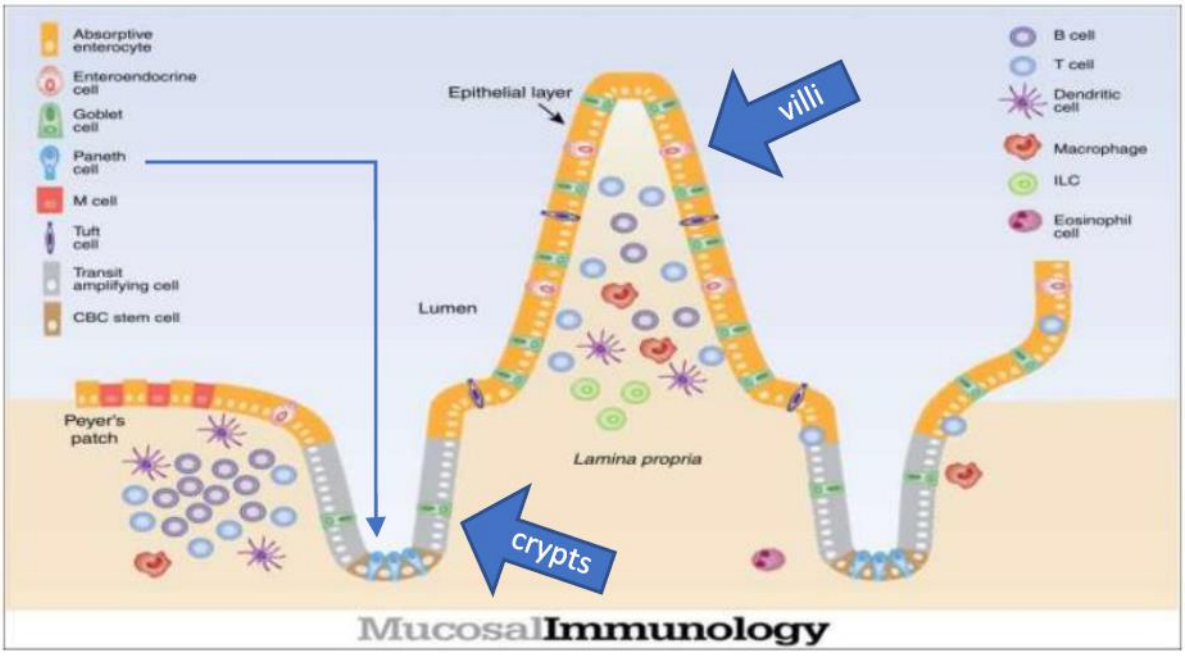
• **Mediators include many cytokines**, act on various tissues (i.e epithelial cells to induce antimicrobial proteins and peptides) and they resist intracellular viral growth. They also can act on other immune cells such as the Innate lymphoid cells (which produce other cytokines) to amplify the immune 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 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.**

- **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, or Yersinia pestis.**

• Abundant intraepithelial lymphocytes (IELs) such as macrophages and dendritic cells present in both organized and scattered tissue of intestine through the surface epithelium of mucosa.

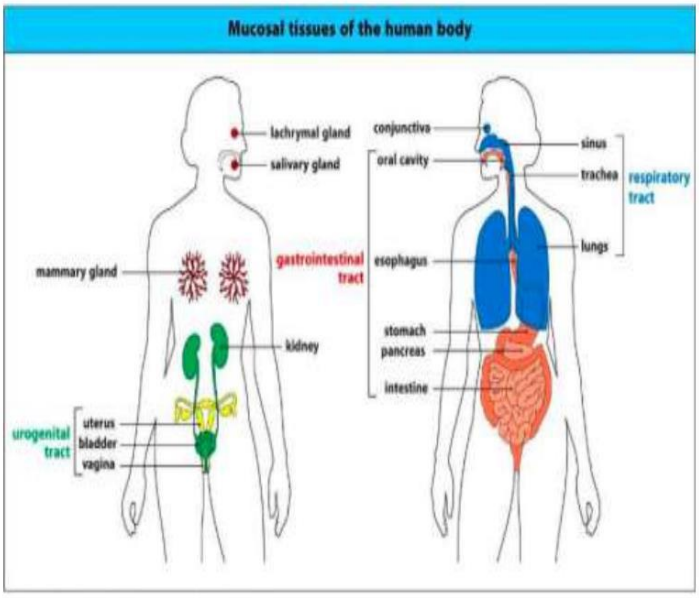
• More than 90% of the IELs in the small intestine are mainly T cells (active T cells ready to act)**, and around 80% of these carry CD8, in complete contrast to the lymphocytes in the lamina propria** (predominantly B cells on the Lamina propria).

❖ Mucosal tissues of the human body:

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

• An enormous area to be protected!

• The Gut mucosal immune system is probably the first part of the vertebrate immune system to evolve due to the need to deal with the vast population of commensal bacteria that co-evolved with the vertebrates.



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

a) The gastrointestinal tract.

b) The upper and lower respiratory tract.

c) The urogenital tract, and the middle ear.

d) It also includes the exocrine glands associated with these organs, such as the conjunctiva and lacrimal glands of the eye, the salivary glands, and the lactating breast.

❖ Distinctive features of the mucosal immune system:

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

Mucosal immune system is bigger, rapidly and frequently encounters a wide range of antigens so it has special anatomical features and mechanisms to act in a different way than the systemic immune system.

❖ Mucosa-associated lymphoid tissues (MALT):

1- 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.

2- The gut-associated lymphoid tissues (GALT) include Palatine Tonsils, adenoids, appendix, lingual tonsils (Palatine, Lingual and other tonsils covered by squamous epithelium and jointly form the Waldeyer's tonsillar 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, Isolated lymphoid follicles -they collect antigen from the epithelial surfaces of the gastrointestinal tract are found throughout the intestine.

3- 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.

4- Tissues above lie in the intestinal wall itself separated from the content of intestinal lumen by a single epithelium layer.

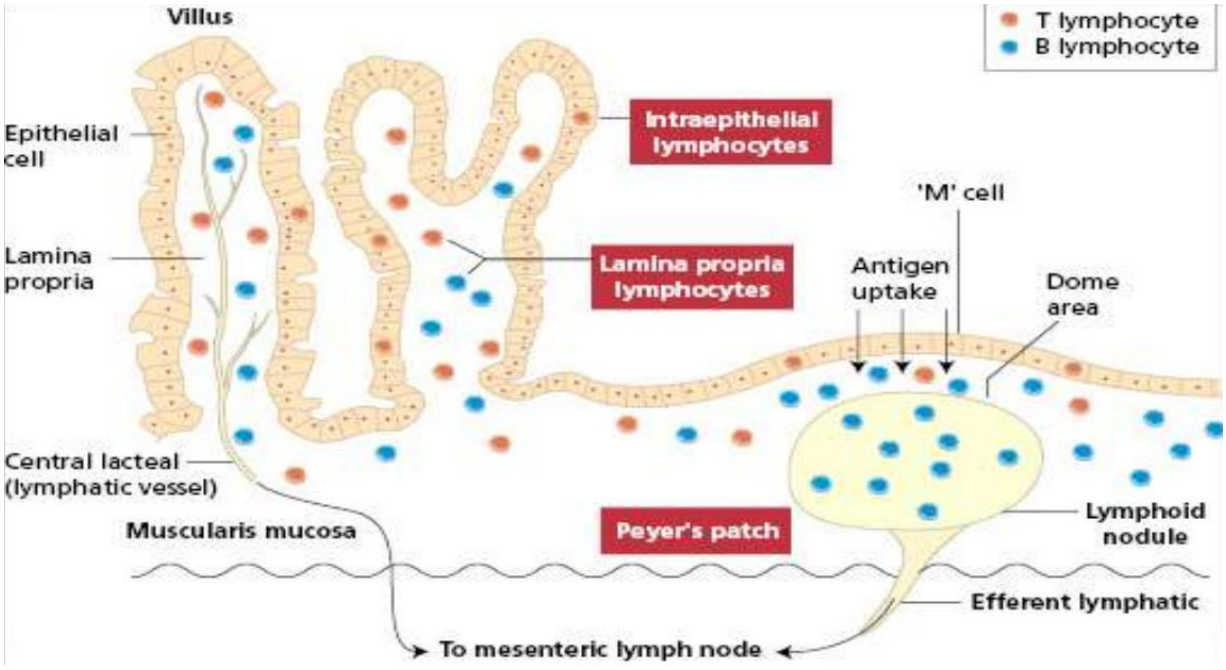
5- Mesenteric lymph nodes and Coda lymph nodes (drains from the gut) are connected to Peyer patches by efferent lymphatic vessels, they are the largest lymph nodes in the body, and together they are the sites for T, B cells antigen presentation, and are also responsible for the induction phase of the immune response. Mucosal immune system is bigger, rapidly and frequently encounters a wide range of antigens so it has special anatomical features and mechanisms to act in a different way than the systemic immune system.

6- Peyer 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).

7- Peyer patches and Mesentric lymph nodes contain discrete T cells area(Brown areas)

8- The main lymphocytes in the isolated follicles is B cells.

❖ Gut Associated Lymphoid Tissue (GALT):



❖ Mucus forms a key protective barrier in the gut:

• GALT promotes clearance of pathogens and separates them from epithelium (a buffer area) to inhibit infection and inflammation, and that's very clear in colon which has large numbers of bacteria.

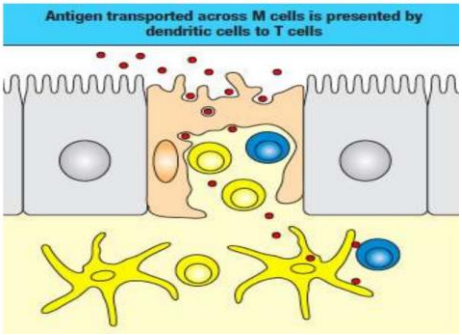
• Goblet cells secrete a heavily glycosylated mucins that oligomerize through disulfide bonds to form mucus. O-linked oligosaccharide modification of the conserved ProlineThreonineSerine (PTS) repeats in the mucin domain maintains the integrity of the epithelial barrier.

• 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 (pre-dominant class in GIT) 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.

❖ Uptake and transport of antigen by M cells:



Antigen present on the mucosal surfaces must be transported from lumen across epithelial layer to be presented to APCs before stimulating mucosal immune system

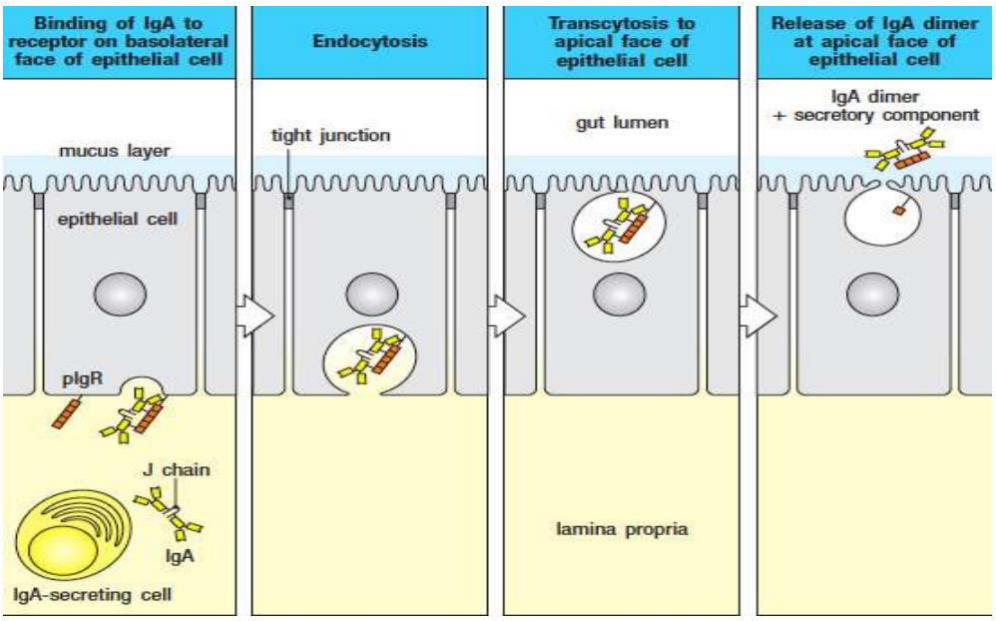
❖ Uptake and transport of antigen by M cells:

1- The intestine has distinctive routes and mechanisms of antigen uptake.

2- 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.

3- 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 → dimer (2 monomers linked by J chain.

- To do its function, 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 (that is called secretory component)

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

MICROBIOTA

❖ What is the microbiome?

1. 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).

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

3. symbiotic relationship with the human host- mutualism (beneficial for both).

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

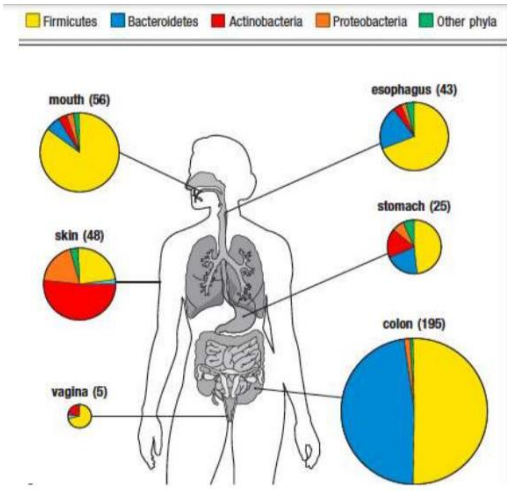
5. 10 trillion human cells, 100 trillion bacteria –Human body (most of them are in the guts especially large intestine, most are confined within the intestinal lumen by a protective layer of the mucous membrane (Mucous membrane plays a buffering role in which separates the microbiome from a direct contact with the epithelium), and they don't cause damage because it can't penetrate mucosa and reach epithelium, unlike pathogenic bacteria which can).

6. >100x more genetic material in microbes than human genome! Ironically, we are more microbes than humans!!

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

❖ Where are they?

- **On the body surface, not inside (blood, deep sterile tissue).**
- **Mainly in the gastrointestinal tract. Flora is different at different sites.**
- **Depends on the environment.**

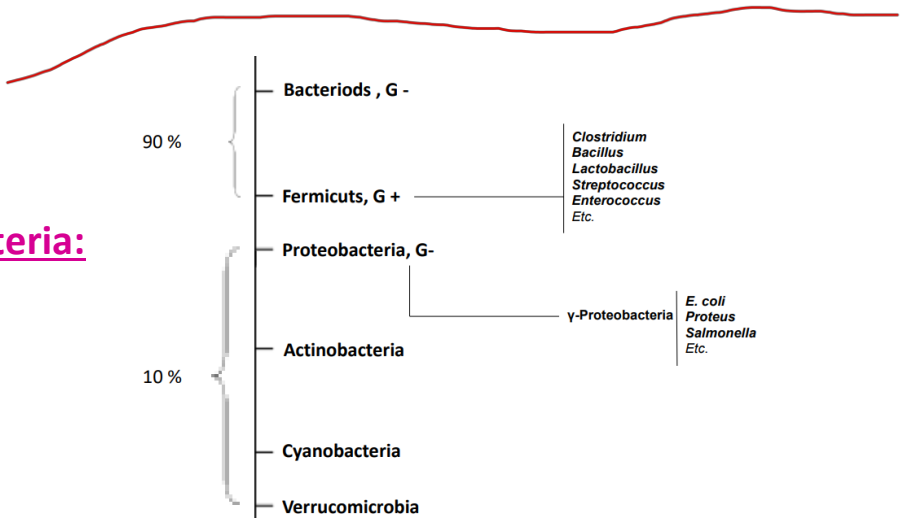


- **Acid, bile and pancreatic secretions hinder the colonization of the stomach and proximal small intestine by most bacteria.**
- **However, bacterial density increases in the distal small intestine, and in the large intestine rises to an estimated 1011–1012.**
- **Blood, urinary tract, CSF are locations that must be sterile.**

❖ Who are they?

- Colon contains the greatest number of different species.
- Frimicutes(i.e. lactobacillus and Clostridium), Bacteroides(i.e. Fragilis which are the common commensal bacteria in the colon), Actinobacteria(i.e. Bifida bacteria which are the common inhabitants in newborns), and Proteobacteria(i.e. E.Coli or Proteus) are the major commensal bacteria.
- **Two main phyla in the intestine: Firmicutes and Bacteroids.**
- **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 the core microbiome of 130 species, plus many others.**
- We classify Skin and Mucous membrane microorganisms to Resident (fixed types of microorganisms, found at a given area, and at a given age, and they re-establish themselves when distributed), and Transient (Non-pathogenic, but potentially pathogenic. Inhabits the skin or mucous membrane for hours, days, or weeks).

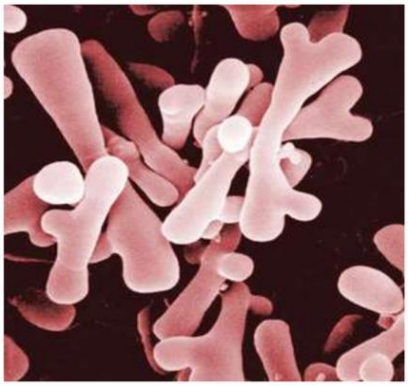
❖ Eubacteria:



Methods to study bacterial microbiota:

1. **Selective plating –strictly anaerobes, needs a community to grow!**
2. **Besides selective plating, we can stain DNA to get total numbers and an idea of population.**
3. **PCR to amplify 16S RNA.**
4. **Fluorescently tagged DNA oligonucleotides to label 16sRNA sequences.**
5. **Microbiota from feces of mouse stained with sybr green DNA stain.**

❖ What influences microbiota?



1. **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).
2. **Nutrition: meat, vegetables.**
3. **Hormones: estrogen, insulin.**
4. **Genetic constitution: receptors on mucosal surfaces.**
5. **Antibiotics: eliminate some which permit others to thrive.** (Antibiotic abuse can lead to overgrowth of clostridium difficile which leads to diarrhea or pseudomembranous colitis).
6. **Foreign objects: valves, catheters.** (bacteria form biofilm around catheters to prevent antibiotics from reaching them)
7. Also when microbiota of GI for example reaches urinary tract it causes UTI (change of location of your own microbiota may cause infection and this depends on personal hygiene

❖ Colonization is immediate and for life:

1. **Acquired at birth, from the environment.**
2. **Ingestion of food, fluids, and inhalation.**
3. **Microbiota established rapidly.**
4. **Mature & Stabilizes later.**

❖ **Food consumption influence microbiota of the small intestine:**

1. Bifidobacterium Spp. Are the primary feces 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.difficile spp.

2. Bifidobacterium are anaerobic, Gram + branched rod-shaped bacteria.

❖ **What do the microbiota do for us?**

(Protection by bacterial interference/ digestion/stimulate host defense)

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

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

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

❖ **What are the harmful effects of microbiota?**

1. Pathogenic potential.

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

b. If host status changes (immunocompromised, nosocomial)

2. 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 diseases. Also can be associated with neural diseases such as autism.

3. Changes in lifestyle

❖ Hygiene theory!

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

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

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

- Increased proportion of Firmicutes (more than bacteroids)
- Related to the ability of microbiota to harness energy from food?

2. Inflammatory Bowel Diseases IBD

- Microbial community imbalances
- increased Proteobacter, depleted Firmicutes and Bacteroidetes

3. Type I Diabetes

- Interaction of intestinal microbes with innate immune system

4. GI Cancers

- H. pylori (only colonizer of stomach but can cause peptic ulcers as well as carcinoma of stomach)

5. Association of various species with colorectal cancer

6. Oral diseases

- Cavities and gingivitis disease Most common infectious disease worldwide

9. Rheumatoid autoimmune disease

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/ bacterial overgrowth)
- Caused by Clostridium difficile
- Common cause of diarrhea after antibiotic use
- Fecal transplants shown to improve outcome
- Treated by oral vancomycin

❖ **Microbiota and the immune system:**

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

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

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. Probiotics

- Live bacteria such as Lactobacilli consumed orally Some protective health benefits (usually it's a component of patient's own microbiome)
- Safe to consume

2. Prebiotics

- Sugars and other foodstuffs used to alter microbiota

3. Immunemodulators

- Inflammation affects microbiota (steroids, immunosuppressants)

4. Antibiotics

- Would increase resistance

5. Phage therapy

- Target specific population (resistance rapidly)

6. Fecal transplants

- Used in C. difficile infections (pseudomembranous colitis).
- May need to deplete current microbiota ϖUse microbiota products

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

8. Need other methods!

THANK YOU!!