GIS 1

MICROBIOLOGY

Online

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Hello poor, miserable med student,
the topic of this sheet is not entirely new,
so maybe that will make things better for you.

الاشياء الموجودة بالسلايدات و ما حكاها الدكتور ملونة بالاخضر و تحتها خط لجماعة الطباعة + اي شي بالبنفسجي (بالخط الصغير برضو لجماعة الطباعة) هو بس عشان اخلي الشيت اطول عليكم 😆ف ازا ما معكم وقت ما تطلعوا عليه ابدا لكن من باب ما دمت ملزما فاستمتع ☆☆

Natural defense of the Gastrointestinal Tract (Microbiome & Immune responses)

Firstly, immune responses:

The gastrointestinal (GI) tract represents the largest surface area in the body (400 m^2, which is 200 times that of skin). The mucosal immune system of the gut is faced with the extraordinary challenge of coexisting with microbiomes (they live symbiotically in the healthy intestines).

So, the GI requires protection from infectious and non-infectious threats continuously introduced during ingestion, as well as prevention of any breach or imbalance caused by the microbiome.

Diarrhea is the most common symptom of GI infections and remains a leading cause of morbidity and mortality worldwide, especially in children. However, it's treatable and preventable.

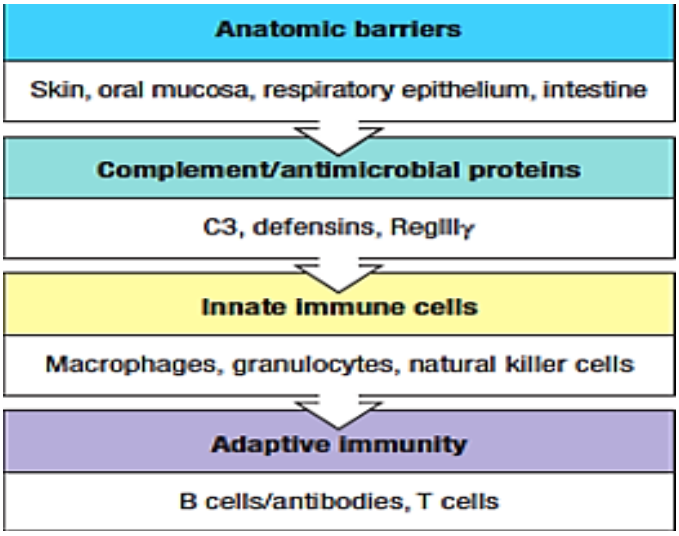
Natural defense:

1. Anatomical & Physiological barrier: skin, oral mucosa and intestinal epithelium, where cells are joined by tight junctions and mucous membranes.
2. Chemical barriers, such as **the acidity of the stomach** (unfavorable condition for many pathogens), complement and antimicrobial proteins (innate immune system).

- Antibacterial enzymes: lysozymes, secretory phospholipase A2 (Paneth cells).
- Antimicrobial peptides: defensins, cathelicidins, and histatins.
- Saliva; contains numerous hydrolytic enzymes (secretory phospholipase A2).
- Antibody production and secretion of **Secretory IgA;** the predominant immunoglobulin in the mucosal immune system (secreted by plasma cells in the mucosal wall).

Protection against pathogens relies on several levels of defense: (about the picture)

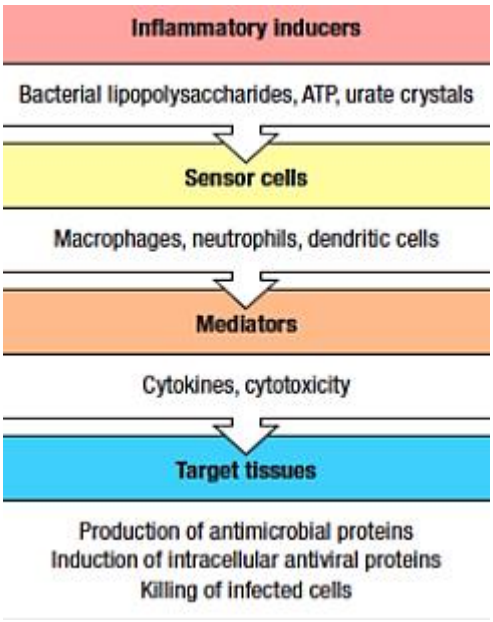
- The first line of defense -against pathogens and commensals- is the thin layer of epithelium. But, the epithelium can be easily breached so it's function is supplemented by cells and molecules of the mucosal immune system.



- The complement system acts as an immediate antimicrobial barrier near the epithelia, so if the epithelia get breached, nearby innate immune cells can mediate a rapid cell-mediated defense.
- If the pathogen overcomes these barriers, slower-acting defenses of the adaptive immune system are brought to the battlefield.

Cell-mediated immunity proceeds in a series of steps:

1. Inflammatory inducers: chemicals of two types:
   a. **PAMPs** (pathogen-associated molecular patterns); they indicate the presence of an invading microbe. e.g. lipopolysaccharide.
   b. **DAMPs** (damage-associated molecular patterns); they indicate the presence of damaged tissue. e.g. ATP, uric acid.
2. Sensor cells; detect the inflammatory inducers by expressing various innate receptors called **pattern recognition receptors (PRRs).**
3. Mediators; they either contribute directly to the defense mechanism or propagate the immune response.



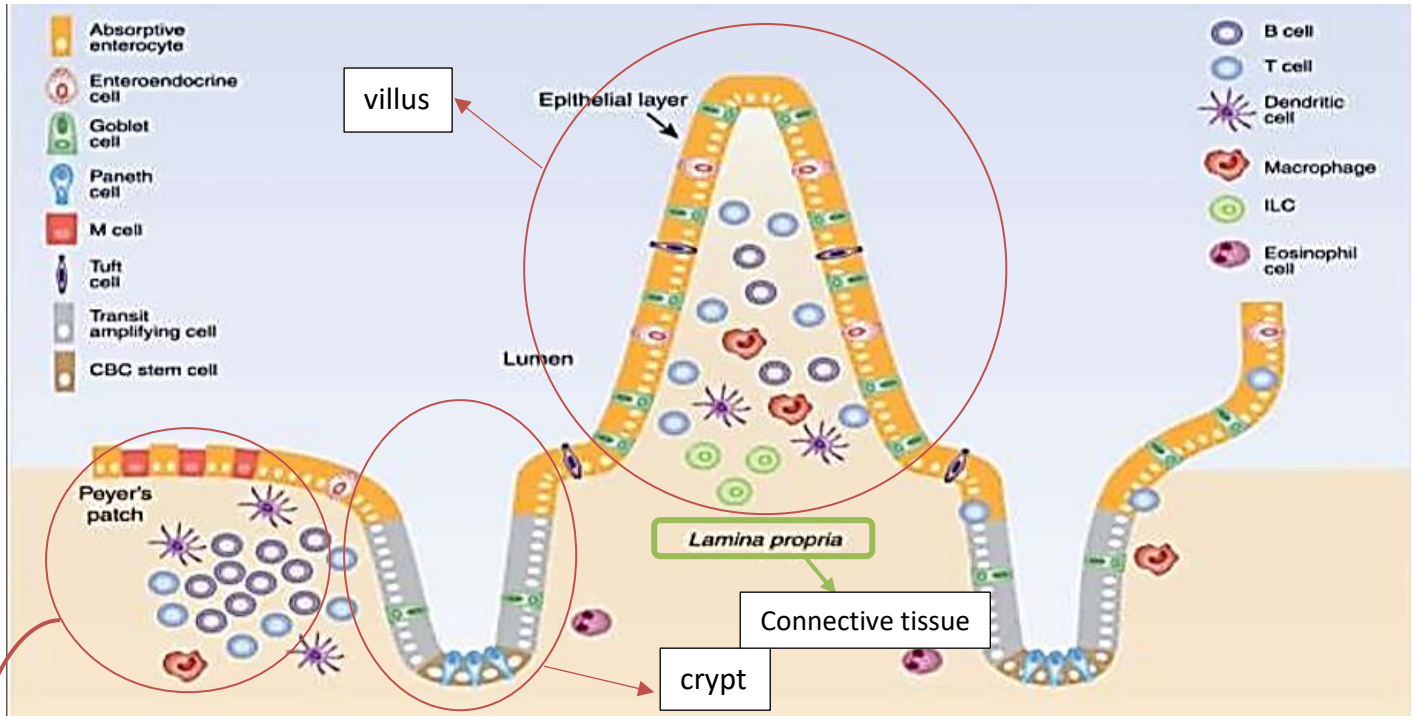
For example, cytokines act on various target tissues such as:

a. Epithelial cells; to produce antimicrobial proteins and peptides, as well as resist the intracellular viral growth.
b. Other lymphoid cells such as innate lymphoid cells which will produce other cytokines that will amplify the immune response.

Epithelial surfaces:

- Most of the enzymatic breakdown of food occurs in the **small intestines**, 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 contain:
  1. Stem cells, which give rise to all the IEC lineages.
  2. Mucus-producing goblet cells found throughout the GI tract.
  3. Paneth cells located in the base of the small intestinal crypts where they secrete antimicrobial molecules.

(See picture in the next page)



****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 includes 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 e.g. Salmonella enterica, Shigella, Yersinia pestis.
- Immune cells like macrophages and dendritic cells are present in an organized manner or scattered through the epithelial tissue.
- Abundant intraepithelial lymphocytes (IELs) present in the intestines.
- 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, where the predominant IELs are B cells.

Mucosal tissues of the human body:

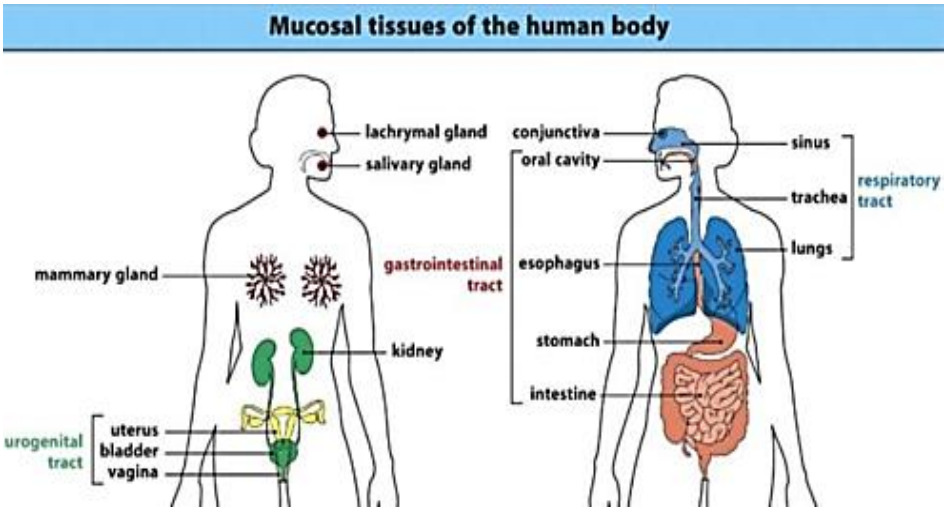
Mucosal surfaces have specialized immune structures that mediate responses to environmental microbial encounters. Granted that there's an enormous area that needs to be protected (areas where there's a continuous encounter with many pathogens).

The Mucosal Immune System:

Comprises the internal body surfaces that are lined by a mucus-secreting epithelium:

- Gastrointestinal tract.
- Upper and lower respiratory tract.
- Urogenital tract, and the middle ear.
- It also includes the exocrine glands associated with these organs, such as the conjunctivae and lacrimal glands of the eye, the salivary glands, and the lactating breast.

**It is thought that the gastrointestinal mucosa was the 1st part of the vertebrate adaptive immune system to evolve. Probably due to the fact that it has a large commensal microorganism population to harbor and live with.



Distinctive features of the Mucosal Immune System:

In contrast to the systemic immune system, the mucosal immune system is bigger with a wider and more frequent range of pathogens encountered. This is reflected in distinctive anatomical features, specialized mechanisms for uptake, and unusual effector and regulatory responses. (e.g. regulatory mechanisms to prevent unwanted immune response to food). ← this is everything the doctor said about this table.

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

Mucosa-Associated Lymphoid Tissues (MALT):

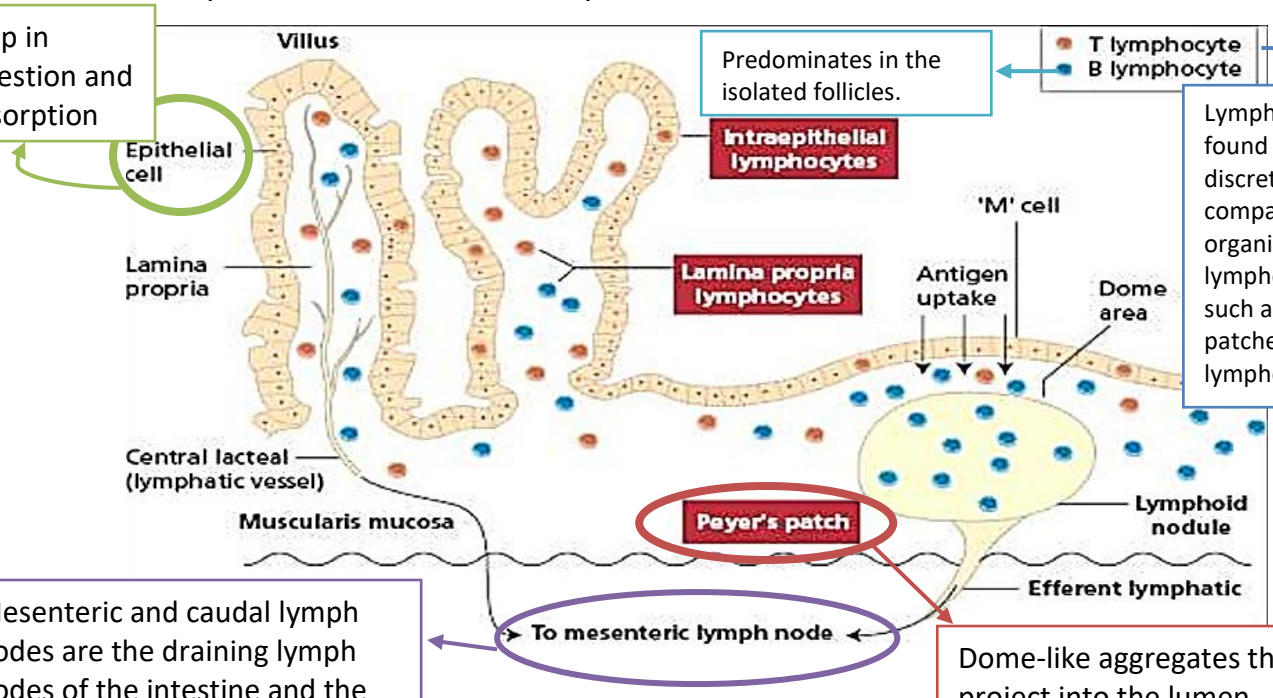
- Collectively, the mucosal immune system is estimated to contain as many lymphocytes as the rest of the body combined, and they form a specialized set of cells obeying somewhat different rules of recirculation from those in the other peripheral lymphoid organs.
- The gut-associated lymphoid tissues (GALT) include the tonsils, adenoids, appendix, and specialized structures in the small intestine called Peyer's patches; Isolated lymphoid follicles that collect antigens from the epithelial surfaces of the gastrointestinal tract.
- In Peyer's patches, which are the most important and highly organized of these tissues, the antigen is collected by specialized epithelial cells called microfolds or M cells.

Gut-Associated Lymphoid Tissue (GALT):

This tissue is separated from the lumen by a single layer of epithelium.

This organized tissue is the site for antigen presentation to T and B cells, and responsible for the induction phase of the immune response.

Help in digestion and absorption



Predominates in the isolated follicles.

Lymphocytes are found in several discrete compartments in organized lymphoid tissues such as Peyer's patches (isolated lymphoid follicles).

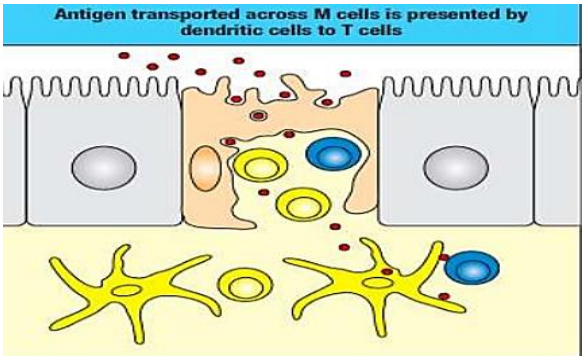
Mesenteric and caudal lymph nodes are the draining lymph nodes of the intestine and the largest lymph nodes in the body, connected to peyer's patches by efferent lymph vessels

Dome-like aggregates that project into the lumen, they're visible to the naked eye and help in initiation of immune response

Mucus forms a key protective barrier in the gut:

- This barrier promotes the clearance of pathogens as well as their separation from the epithelium (buffering), thus, inhibiting infection and inflammation. However, some pathogens can work their way to overcome this barrier.
- The function of the mucous layer in the colon is important in explaining our ability to harbor large numbers of bacteria in our gut.
- Goblet cells secrete heavily **glycosylated mucins (type2) that forms large net-like polymers** through disulfide bonds to form mucus. O-linked oligosaccharide modification of the conserved Proline-ThreonineSerine (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 scaffold 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.

Uptake and transport of antigens by M cells: بدنا ندخّل اشياء من برا لجوّا

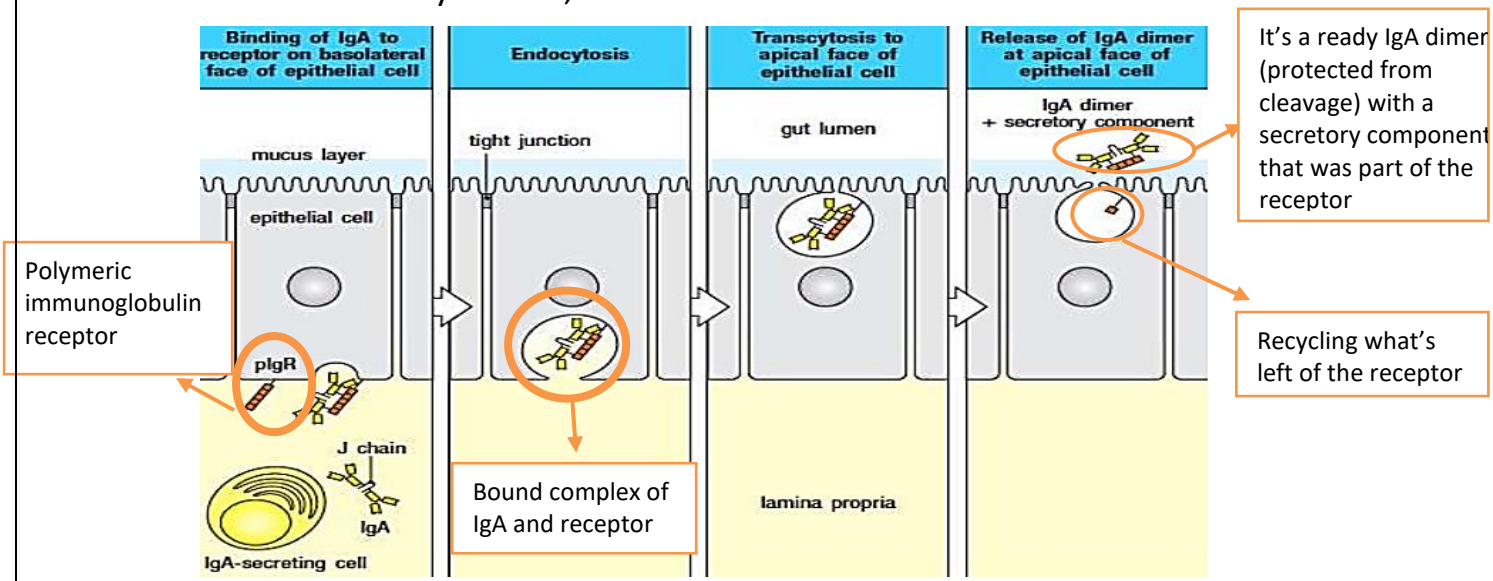


- Antigens have to cross the epithelium to reach the immune cells. So, Intestinal cells have their own way of uptake and antigen presentation.
- The M cells have a convoluted basal membrane that forms pockets within the epithelium, which helps in close contact with immune cells.
- For several bacteria, this may involve specific recognition of the bacterial FimH protein found in type 1 pili by a glycoprotein (GP2) on the M cell. Local transport of this material 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: بدنا نطلّع من جوّا لبرا -pay attention to the steps

the IgA is of two types:

a. Monomer: found in the blood
b. Dimer: linked by J-chain, found in mucous membranes



Secondly, Microbiota: Agents of health and disease

Now, are you ready for the adventure of your life? We're going on an exploration journey through the body and I'll be your guide. So gear up and let's go: we'll begin our journey in the oral cavity, where you can see some microbiota communities. Say hello.

What is the microbiota/ microbiome (also called commensals)?

- Cute little microbial communities that constantly colonize the skin and mucosal membranes of healthy individuals, (oral mucosa, conjunctiva, GI mucosa). (used to be called "normal flora")
- Microbiota **in and on** our bodies include bacteria, viruses, and eukaryotes (fungi, archaea).

- There's a symbiotic relationship between the microbiome and the human host (living together), some even consider it a mutualistic relationship, where both parties are benefitted.
- One gram of feces contains > the world's population.
- Don't misjudge them! There are many thousand species, yet only about 100 are pathogens.

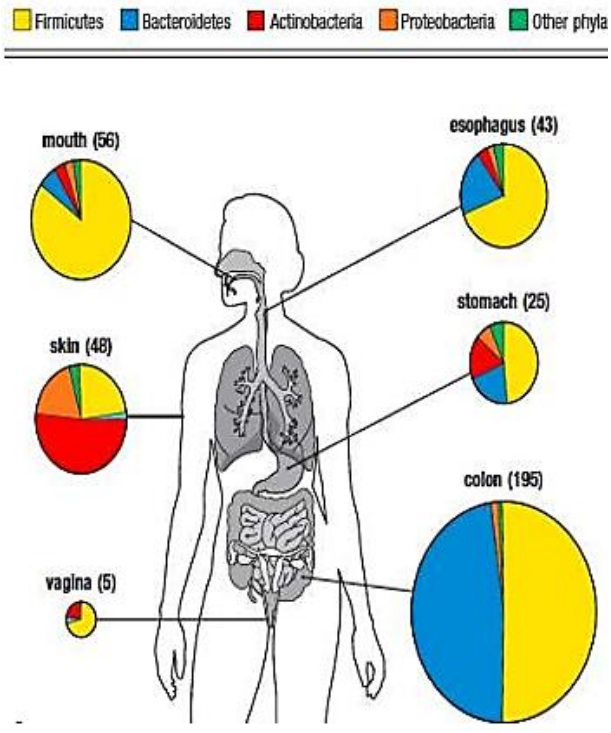
We're inside the oral cavity, there's an upcoming waterfall (pharynx to esophagus) so hold on tight!!!!

You are your microbes!! Our body contains 100 trillion bacterial cells vs. 10 trillion human cells. >100x more genetic material in microbes than human genome! Ironically, we are more microbes than human!! So be humble, you're just a sad little bacterium.

Where are they?

- On body surfaces that are in contact with pathogens directly or indirectly, but not inside the blood or deep sterile tissues.
- Mainly found in the gastrointestinal tract. The more distal you go the more flora you find. (Bacterial density increases in the distal small intestine, and in the large intestine it rises to an estimated 10^{11}–10^{12}).
- They're found within the lumen. The mucous layer plays a key role in buffering the microbiota not to reach the epithelium. In contrast to the pathogenic bacteria, which penetrates this barrier and reaches the underlying tissues.
- It depends on the environment.
- Acid, bile and pancreatic secretions hinder the colonization of the stomach and proximal small intestine by most bacteria.

Wow! What a ride!! is everybody okay? The waterfall started at the oral cavity, pharynx, esophagus reaching here to the stomach, which are all colonized with microbiota.



- Different sites contain different flora as the picture indicates.
- Note that the colon contains the largest number of bacteria (size of the chart).
- The body contains 4 phyla of bacteria:
  a. Firmicutes.
  b. Bacteroides (anaerobes common in colon).
  c. Actinobacteria (bifidobacteria; commonest in newborns).
  d. Proteobacteria.

Who are they?

We drifted to the shore, coughing our hearts out when we saw two types of commensals glaring at us:

1. Resident commensals: these are the tribe leaders, regularly found in a specific area at a specific age. If they are disturbed, they re-establish themselves. (they look like giant snakes with two heads, if you try to cut off one head it grows back immediately).

2. Transient commensals: they're not harmful, but they're potentially pathogenic. They stay for hours, days or weeks. (tourists).

Two figures approached us: the first was a long, red, rod-shaped bacterium, extending his hand he said: "I'm bacteroid, please don't panic we won't hurt you". I shook his hand and then the other purple bacterium started saying: "and I'm firmicute, we are the leaders here in the intestines, let us show you around".

They led us through the intestines, introducing us to the other phyla there. Turns out the firmicute had kids, some of them were rods like clostridia and lactobacillus. Some were round like enterococcus. We were approaching a red group when suddenly one of their kids rushed towards me saying: hello, my name is E-coli, have you met my phylum the proteobacteria? I have two siblings; salmonella and proteus.

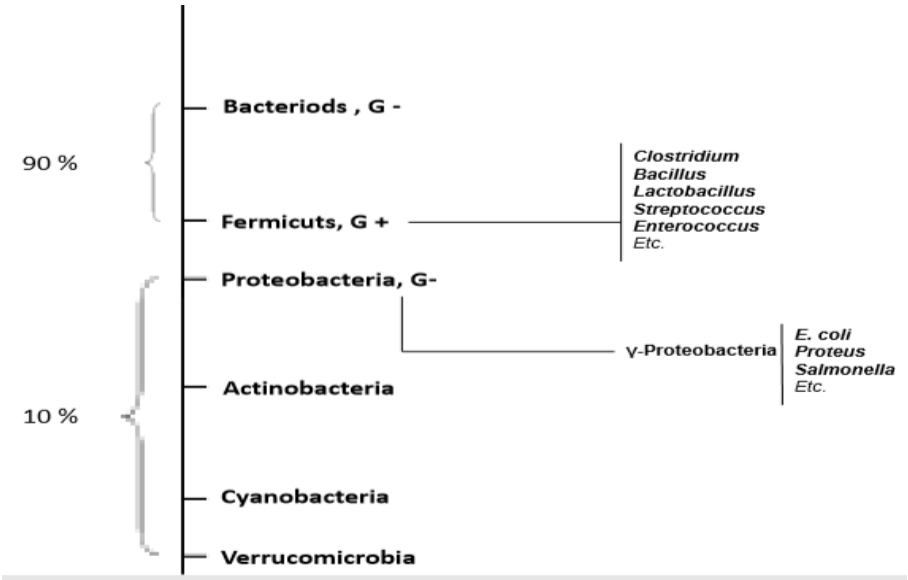
Two main phyla in the intestines: **Firmicutes and Bacteroides**.

At the phyla level, composition is similar between humans and mice.

Many individual variations, many species.

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

Eubacteria (in intestines): -pay attention to the subtypes also-



After our tour through the intestines, the microbiota took us to their camp in the large intestines. We sat around the fire and they told us their story:

Methods to study bacterial microbiota:

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

What influences the microbiota?

They are dynamic and subject to many changes throughout the host's life in response to many factors including:

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

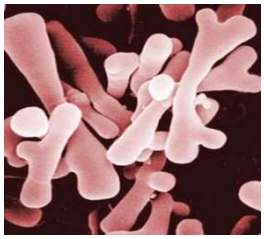
- Nutrition: meat, vegetables.
- Hormones: estrogen, insulin.
- Genetic constitution: receptors on mucosal surfaces.
- Antibiotics: eliminate some which permit others to thrive.
- Foreign objects: valves, catheters.

Regarding the way of birth and how it affects microbiota:
Vaginal delivery introduces the vaginal microbiota to the baby. E.g. lactobacilli, bifidobacteria which are normally present in the mother's vagina.
On the other hand, delivery through C-section introduces skin flora to the baby e.g. S.aureus

When and how do we acquire our Microbiota?

Colonization is immediate and for life, but it stabilizes and matures functionally and compositionally later in life.
Before birth, the intestines are sterile. The microbiota is acquired at birth; during delivery. Also, the environment, ingestion of food, fluids and inhalation all determine the early microbial profile.
e.g. breast-fed babies are thought to have bifidobacteria as a predominant commensal.

Food consumption:



- Influences microbiota of the small intestine.
- Bifidobacterium spp. (anaerobic, Gram +, branched, rod-shaped) are the primary feces inhabitants shortly after birth.
- As the child shifts from mother's milk to solid food, the microbiota shifts to a more mixed population including other anaerobic bacteria; C.difficile.

What does the Microbiota do for us? (functions)

1. Microbial antagonism (protection):
   Indirectly: It plugs up sites, consumes nutrients; competing for space and nutrients.
   Directly: produces inhibitory substances that affect pH and oxygen, and antimicrobials like bacteriocin and lactic acid.
2. Nutritional benefits, Vitamin K, B12, Steroid metabolism (breaks down bile acids), Organic acid production, Food breakdown.
3. Stimulate and enhance host defenses; we need normal flora to develop a normal immune system.

I asked: 'can the microbiota be harmful?' Everybody's eyes darted to me. The leader shot me a look that lasted ages as if he's evaluating if we were trustworthy or not. Finally, he started talking:

What are the harmful effects of Microbiota?

1. They're **potentially pathogenic,** meaning that they can cause infections if:
   a) Introduced into other body sites - through trauma for example - especially if these sites were sterile, e.g. Urinary tract infections, septic shock.

b) If the host status changes (immunocompromised, nosocomial)

c) 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.

e.g. antibiotics abuse can lead to overgrowth of C.difficile in the colon causing pseudomembranous colitis.

2. They can change the health status, mental status, and they can even change your mind!
3. They can switch to a range of serious diseases like cancer, cardiological problems, metabolic diseases, allergy, obesity, autoimmune diseases, or even autism.
4. Changes in lifestyle.
5. Gaseous by-products, Fermentation by-products: Hydrogen disulfide, methane 300 ml/day in gas produced.

I nodded when he finished talking, of course everybody has a dark side I understand that. But before I could process what the leader had just told me, a tiny blue-green alga I recognized from the cyanobacteria phylum asked enthusiastically:

'My grandmother used to tell me stories about our ancestors, and there's a myth called the hygiene theory! Do you want to hear it?' Of course she wasn't waiting for an answer and immediately started talking:

Hygiene theory:

It states that a lack of early childhood exposure to infectious agents or symbiotics is linked to defects in the development of immune tolerance. Thus, increasing the risk for diseases especially autoimmune ones.

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).

That was an interesting one, proves that caution and over cleanliness is not always beneficial. The little alga yawned and waved good night to all of us. Everybody then followed suit. I was about to go to my tent to sleep when someone behind me said: 'don't you wanna hear about the only thing our leader didn't tell you?' I turned around and saw the purple round streptococcus, and I was intrigued since she was the only one who was silent all night. 'follow me' she said, and disappeared into the mesentery. I tried to follow her but it was too dark in there,but I could hear her anyway so I just stood there listening.

'We've been through many wars with the humans' she started.

Microbiota and disease:

1. Obesity: related to a lower diversity of microbiota with increased enzymes; efficient digestion and harvesting calories and increased proportion of Firmicutes.
   Related to the ability of the microbiota to harness energy from food?
2. Inflammatory Bowel Diseases IBD:
   Microbial community imbalances
   Increased Proteobacteria and depleted Firmicutes and Bacteroides

3. Type I Diabetes: Decreased gut microbiota diversity.
   Interaction of intestinal microbes with the innate immune system
4. GI Cancers: H. pylori
5. Association of various species with colorectal cancer
6. Oral diseases: 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: A swelling in the colon and one of the common causes of diarrhea. Follows antibiotic treatment (which alters gut microbiota) caused by C.difficile. Fecal transplants were shown to improve outcomes.
   'It's not really well understood why and how we got into these wars. But we did and we're not allowed to talk about it anymore. It causes conflict, and even hatred'. She finished. I was so overwhelmed. 'but the humans must know the importance of their microbiota, what happens if your balance is disturbed? I asked. 'they have ways to restore us back' she answered.

To manipulate Microbiota:

1. Probiotics: Live bacteria such as Lactobacilli consumed orally with protective benefits (improves or restores the microbiota). They're safe but in rare cases, they may cause bacterial-host interactions or unwanted side effects.
2. Prebiotics: Sugars and other foodstuffs used to induce the growth or the activity of beneficial microbiota.
3. Immunomodulators: Inflammation affects microbiota.
4. Antibiotics: over-use would increase resistance.
5. Phage therapy: Target specific populations (resistance rapidly)
6. Fecal transplants used in C. difficile infections. May need to deplete current microbiota
7. Use microbiota products
8. A bacterial polysaccharide from Bacteroides fragilis affects T cell population and Th1/Th2 balance
9. Need other methods!

Microbiota and the Immune System

- Recently realized that the microbiota plays a key role in immune system development.
- Germ-free (microbiota free) animals have a poorly developed immune system.
- Activation of Toll-like receptors (TLRs) needed for development.
- Segmented Filamentous Bacteria (SFB) are needed for Th17 cells. Critical T cell lineage Germ-free mice lack Th17 cells Antibiotics affect Th17 levels.
- Very recently shown that T-reg cells are affected by microbiota.

I jumped with fright when I heard her voice next to my ear whispering: ‘now you know everything about us, except…’ she paused, my heart was racing. ‘you didn’t ask what happened to your fellow explorers who came here before you’ she continued. And then a laugh shrieked from her throat that rang in my ears.

The end.