

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

By : Nader Alaridah MD, PhD

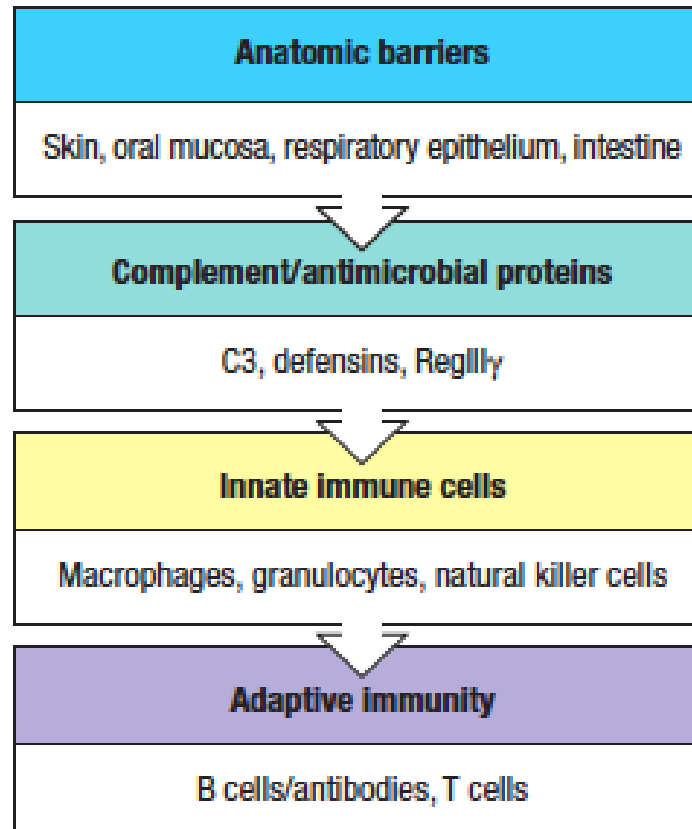
Key facts

- 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.
- 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 microbioms and simultaneously preventing a breach in a single layer of epithelial cells.

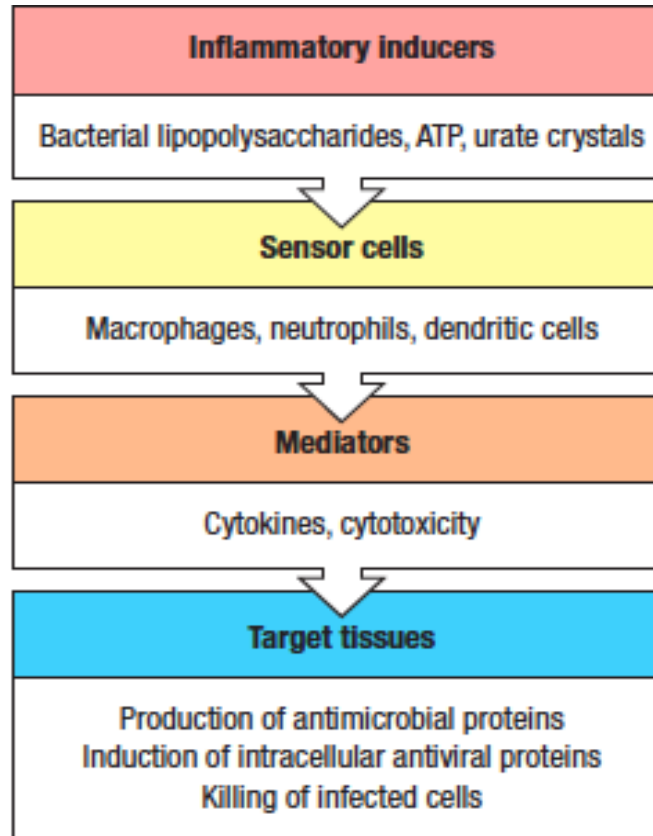
Natural defense

- ❖ Anatomical & physiological barrier : skin (epithelium) where cells joined by tight junctions and mucous membrane.
- ❖ chemical barriers such as The acidity of the stomach , complement and antimicrobial proteins.
 - 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 Sec. IgA.

Protection against pathogens relies on several levels of defense

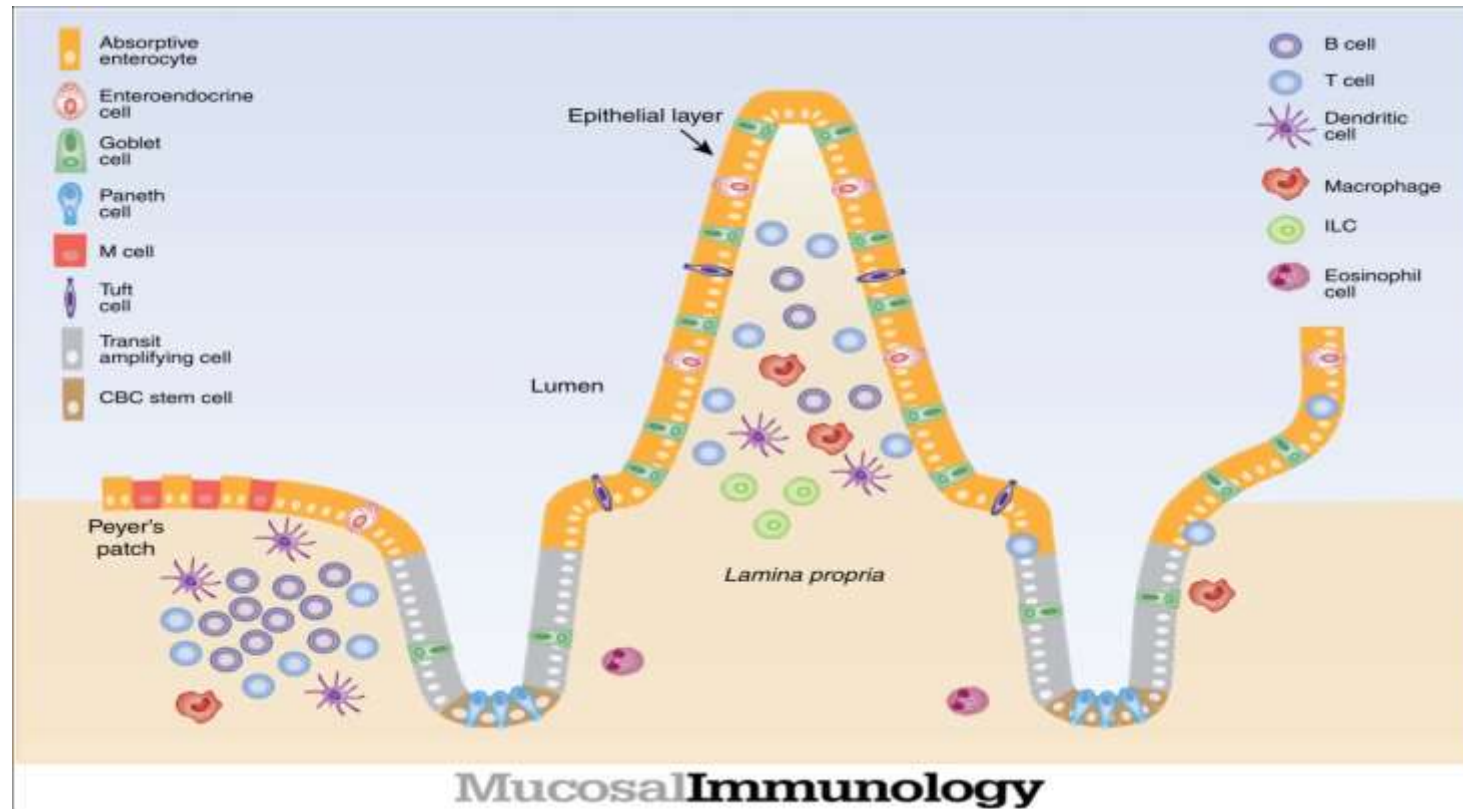


The immune system is activated by inflammatory inducers that indicate the presence of pathogens or tissue damage



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.

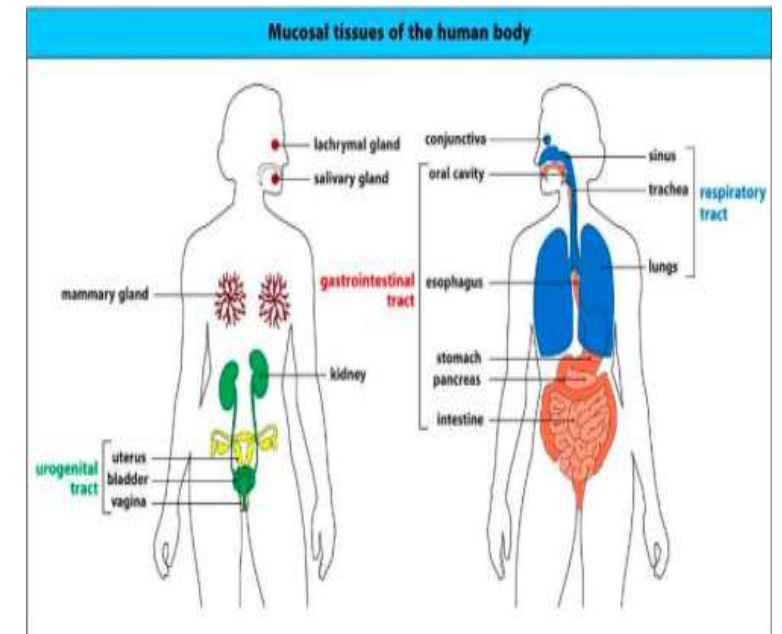


The intestinal epithelium is a unique compartment of the immune system

- 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* *Yersinia pestis*.
- Abundant intraepithelial lymphocytes (IELs) present in intestine 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.

Mucosal tissues of the human body

- 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—
 - ❖ the gastrointestinal tract .
 - ❖ the upper and lower respiratory tract.
 - ❖ the urogenital tract, and the middle ear.
 - ❖ It also includes the exocrine glands associated with these organs, such as the conjunctivae and lacrymal glands of the eye, the salivary glands, and the lactating breast



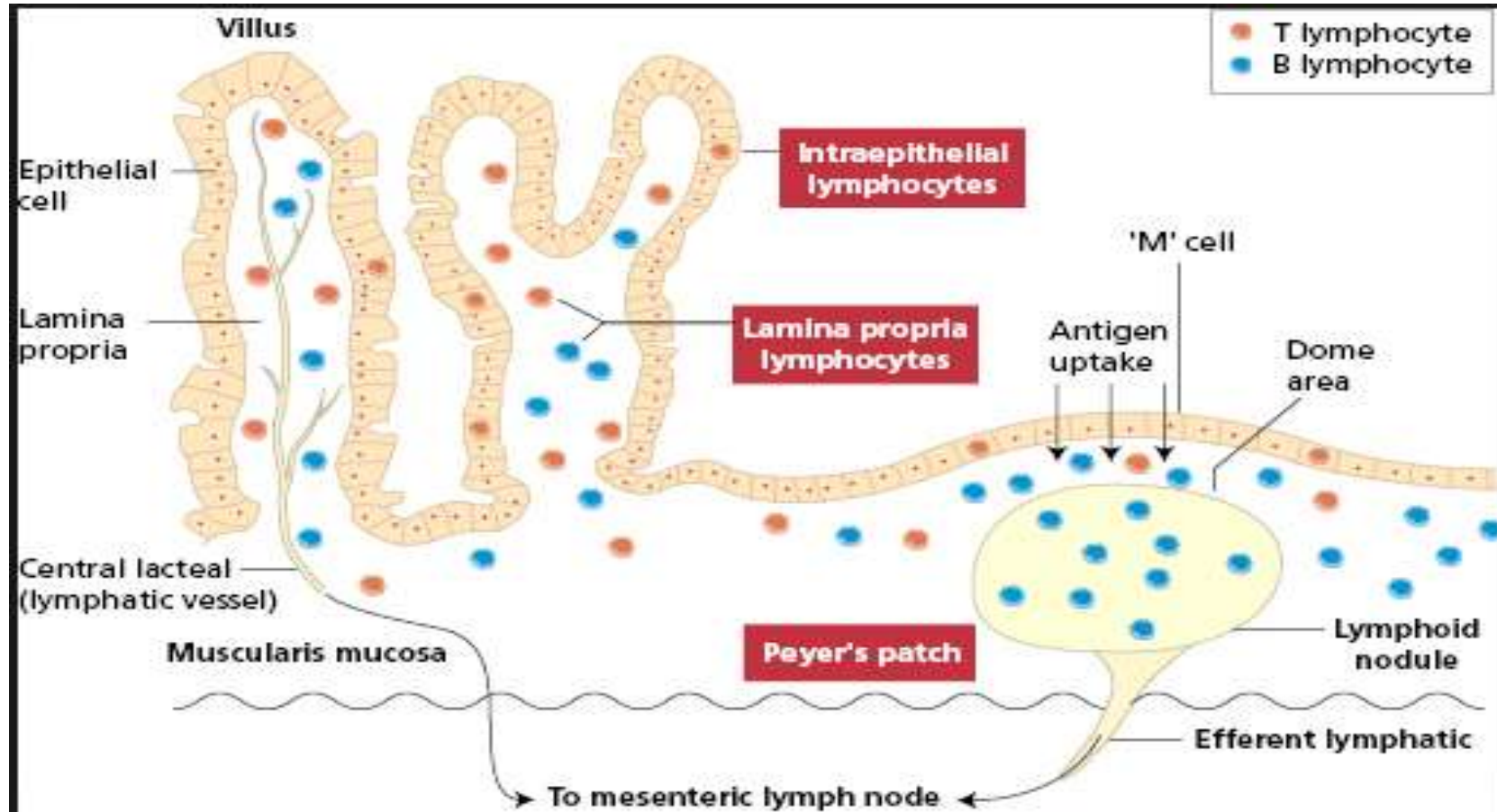
Distinctive features of the mucosal immune system

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 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, 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.
- 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.

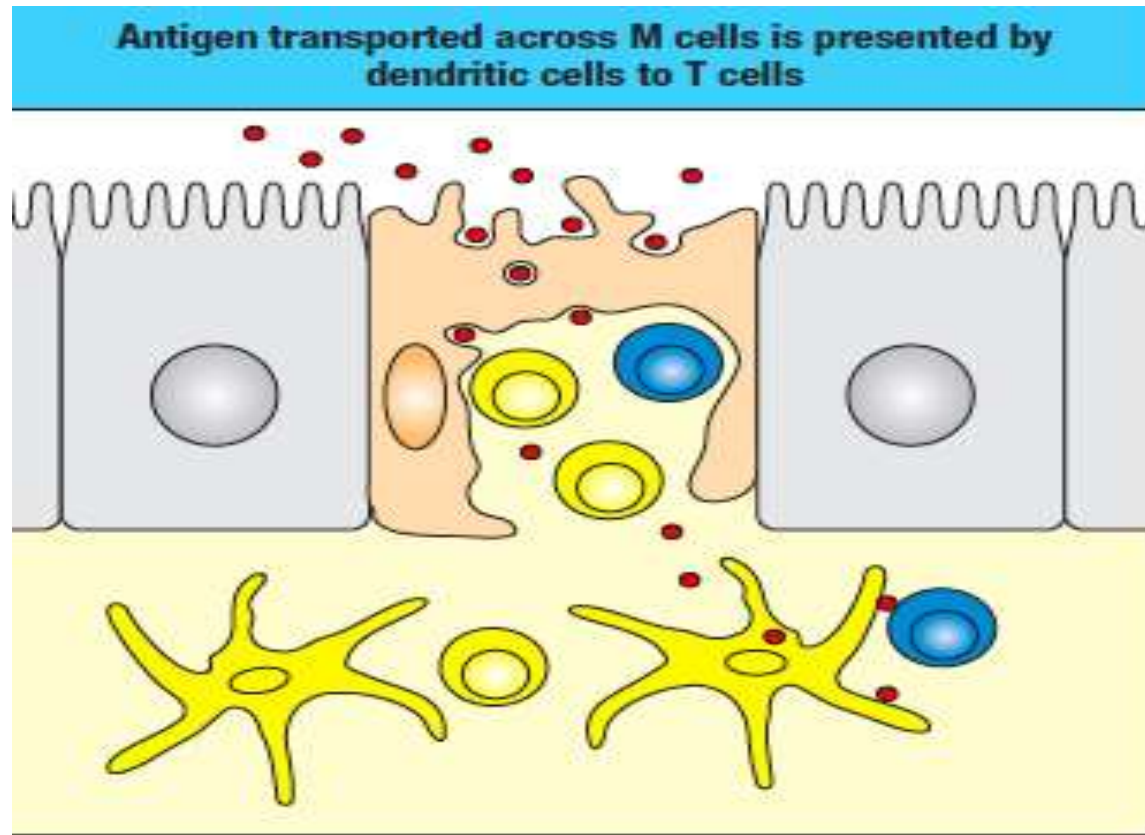
Gut Associated Lymphoid Tissue (GALT)



Mucus forms a key protective barrier in the gut

- 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.
- 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.

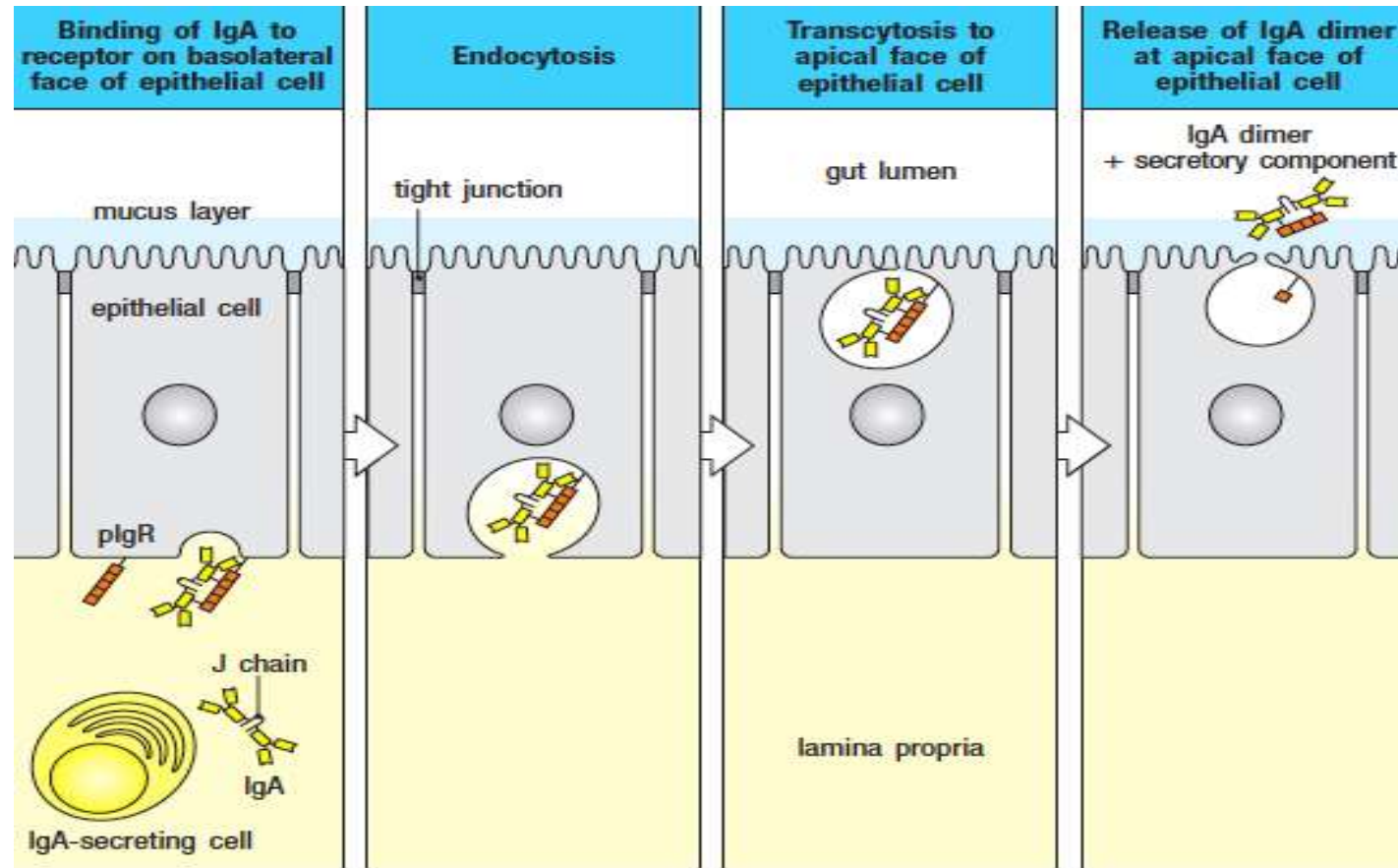
Uptake and transport of antigen by M cells



Uptake and transport of antigen by M cells

- 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



Microbiota :Agents of health and disease

- A general overview
- Several aspects of microbiota
- Various contribution in health and disease
- Potential manipulation
- Interface with the immune system and its development

What is the microbiome?

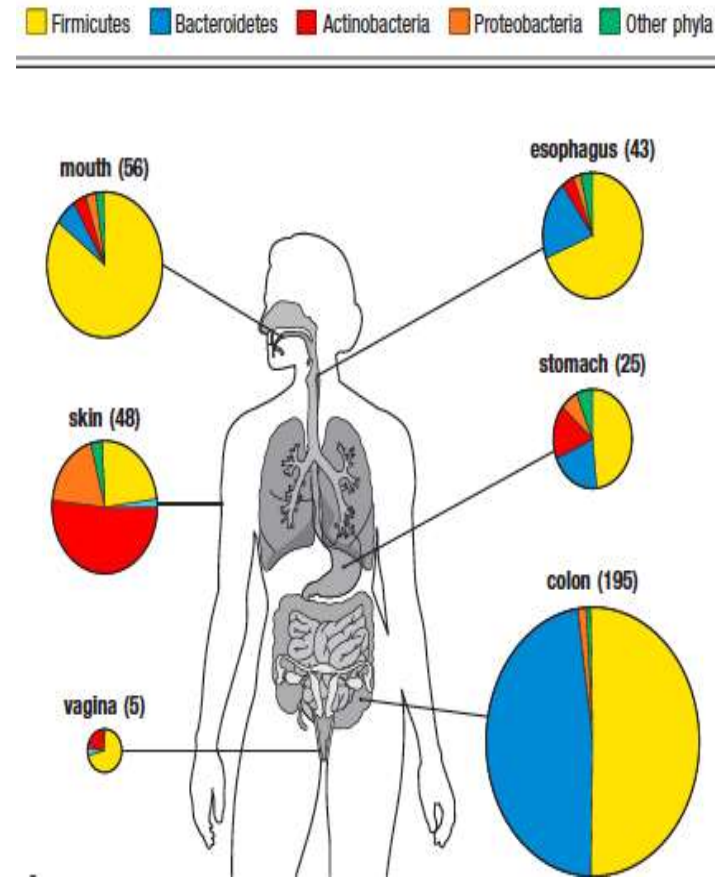
- Not all microbes are pathogens. Many tissues, especially the skin, oral mucosa, conjunctiva, and gastrointestinal tract, are constantly colonized by microbial communities—called the microbiome ; used to be called “normal flora”.
- All the microbes (microbiota) in and on our bodies Includes bacteria, viruses, and eukaryotes.
- symbiotic relationship with the human host- mutualism.
- Vast numbers on body sites 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
- >100x more genetic material in microbes than human genome! Ironically ,we are more microbes than human !!
- Many thousand species (yet only about 100 are pathogens)

Where are they ?

- On body surface, not inside (blood, deep sterile tissue) .
- Mainly in gastrointestinal tract . Flora 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 10^{11} – 10^{12}



Who are they?

- Two main phyla in intestine : Firmicutes and Bacteroids .
- At phyla level composition is similar between humans and mice .
- Much individual variation, many species .
- Shared by humans, Thought to be core microbiome of 130 species, plus many others .

Eubacteria

90 %

Bacterioids , G -

Fermicuts, G +

Clostridium
Bacillus
Lactobacillus
Streptococcus
Enterococcus
Etc.

Proteobacteria, G-

γ -Proteobacteria

E. coli
Proteus
Salmonella
Etc.

10 %

Actinobacteria

Cyanobacteria

Verrucomicrobia



Methods to study bacterial microbiota

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

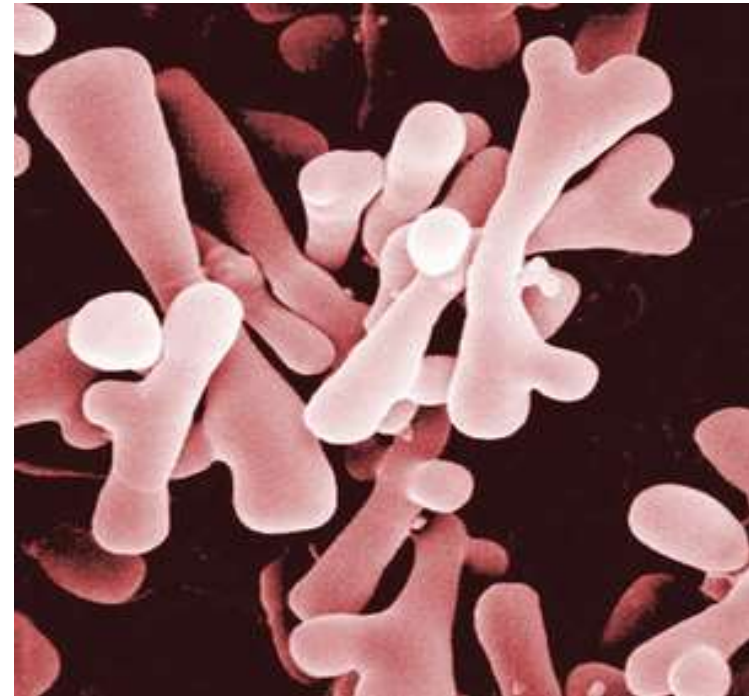
Colonization is immediate and for life

- 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 mix population – other anaerobic bacteria *Cl.difficil* spp.

- Bifidobacterium are anaerobic ,
Gram + branched rod shaped bacteria



What do the microbiota do for us?

- Microbial antagonism –space and nutrients competition ! Plugs up sites, consumes nutrients, produces inhibitory substances, affects pH and oxygen .
- Nutritional benefits Vitamin K, B12 , Steroid metabolism (breaks down bile acids) Organic acid production , Food breakdown .
- Stimulate and enhance host defenses, Need normal flora to develop normal immune system.

What are the harmful effects of microbiota?

- Pathogenic potential
 - If introduced into other body sites – Urinary tract infections, septic shock, etc.
 - 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
- Changes in lifestyle

Hygiene theory !

- 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) .

Microbiota and disease 1

❑ Obesity

- Increased proportion of Firmicutes
- Related to ability of microbiota to harness energy from food?

❑ Inflammatory Bowel Diseases IBD

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

Microbiota and disease 2

☐ Type I Diabetes

- Interaction of intestinal microbes with innate immune system

☐ GI Cancers

- *H. pylori*

☐ Association of various species with colorectal cancer

☐ Oral diseases

- Cavities and gingivitis disease

Most common infectious disease worldwide

Microbiota and disease 3

❑ Allergy-like (atopic) diseases

Eczema, allergies, asthma

Hygiene hypothesis

Induction of tolerance (early exposure)

Antibiotic treatment, C section increase rates of asthma

❑ Pseudomembranous colitis

Follows antibiotic treatment (which alters gut microbiota) Caused by

Clostridium difficile

Fecal transplants shown to improve outcome

Microbiota and the immune system

- Recently realized microbiota plays a key role in immune system development .
- Germ free (microbiota free) animals have 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 Treg cells affected by microbiota

To manipulate Microbiota 1

- ❖ Probiotics

Live bacteria such as *Lactobacilli* consumed orally Some protective benefits

- ❖ Prebiotics

Sugars and other foodstuffs used to alter microbiota

- ❖ Immunomodulators

Inflammation affects microbiota

- ❖ Antibiotics

Would increase resistance

To manipulate Microbiota 2

- ❖ Phage therapy
Target specific population (resistance rapidly)
- ❖ Fecal transplants
Used in *C. difficile* infections
May need to deplete current microbiota
- ❖ Use microbiota products
- ❖ A bacterial polysaccharide from *Bacteroides fragilis* affects T cell population and Th1/Th2 balance
- ❖ Need other methods !

The End .