Seminar 3

Bad Bugs – Infection

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Learning in Retirement
Where do you find bacteria

**Bacteria prefer**

- Mucosal surfaces
- A place to hide
- Lots of nutrients
- Specific conditions
- Limited washing/flushing

**Bacteria Avoid**

- Epithelial surfaces
- Open areas
- Low nutrient areas
- Unfamiliar conditions
- Washing/flushing
Digestive Tract
AKA – “The Gut”

- 30 feet long
- 400 m$^2$ of surface area
- 1,000,000,000,000,000 individual bacteria
- Contains a nice warm mix of nutrients
- Plenty of places to hide
- Several different environments along the route
Resident Flora
Different Segments Have varying flora

**Oral Cavity;** over 700 various species of bacteria
- Primarily on tongue, tooth + gum surfaces
- Saliva is a poor medium: ↓ nutrients ↑ antibacterials

**Stomach;**
- extremely acidic, kills most bacteria that travel though it
- some can live or pass through
  - More bacteria = greater chance of survival
  - Helicobacter, Proteobacteria, Bacteroidetes, Actinobacter, Fusobacteria

- i) Use outer slime layer   
  ii) bury in stomach mucus  
  iii) reduce pH inside the cell   
  iv) spores   
  v) other
Surviving an Acid Trip

Researchers discovered that some species have chaperone proteins that become active in acidic environments and protect periplasmic proteins.

How bacteria survive an acid trip

Karan S. Hingorani and Lila M. Gierasch
PNAS April 2, 2013 110 (14) 5279-5280; https://doi.org/10.1073/pnas.1303297110
Helicobacter Pylori

Spiral, microaerophillic, lophotrichous, Gram negative, present in ~50% of people, asymptomatic in 80-90% of cases

Neutralizes acid in its environment, buries in mucosa

Peptic ulcers are open sores in the mucosa of the stomach or duodenum that don’t heal, acid and inflammation can perpetuate ulcers

- cancer, physical damage, overuse of drugs (painkillers), stress, and excessive alcohol use are associated with the formation of peptic ulcers
- In mid-late 1980s, studies showed that H. pylori was present in 80-90% of ulcers. Major contributor to gastritis – (stomach inflammation) + gastroenteritis (inflammation of stomach/intestines)
Gastric vs Duodenal ulcers

Duodenal Ulcer (DU) vs Gastric Ulcer (GU)
Resident Flora
Different Segments Have varying flora

Small Intestine: Difficult to colonize because of peristalsis, bile, pancreatic juice.

Acid is neutralized.

Primarily aerobic/facultative anaerobic, Gram positive bacteria – Use polysacchide walls or teichoic acid to bind mucosa.

*Enterococcus faecalis* and lactobacilli dominate

Diet specific: More Meat = ↑ *Bacterioides* ↓ *Lactic acid Bacteria*

More Veggies = ↓ *Bacterioides* ↑ *Lactic acid Bacteria*

Large Intestine/Colon: Slow flow, dense bacterial concentration, *primarily anaerobic* and Gram negative – use attachment pilli to bind mucosa. *Bacteroides*, and bifobacteria dominate. *E.coli* present in lower amounts.
Gram + : Use polysaccharide walls or teichoic acid to bind the mucosa

Gram - : Use pili to bind
Benefits of the Resident Flora

Protective

• presence in small intestine prevents pathogens from dominating – competition, and inhibition by endo/exo-toxins

• Normal bacteria stimulate the growth of the intestinal lining

Structural

• makes up part of the intestinal barrier (mucosal)

• critical in the natural development of the immune system

Metabolic

• resident flora of the small intestine protect the host by metabolizing carcinogens in dietary foods.

• provide the host with synthesized vitamins, such as biotin and folate – Synthesize vitamin K

• important for the muscular activity of small intestine. Without bacteria, there is reduced muscular activity

help metabolise complex sugars
Benefits of the Resident Flora

**Good and Bad Bacterial Flora**

- **BIFIDOBACTERIA**
  - The various strains help to regulate levels of other bacteria in the gut, modulate immune responses to invading pathogens, prevent tumour formation and produce vitamins.

- **ESCHERICHIA COLI**
  - Several types inhabit the human gut. They are involved in the production of vitamin K2 (essential for blood clotting) and help to keep bad bacteria in check. But some strains can lead to illness.

- **LACTOBACILLI**
  - Beneficial varieties produce vitamins and nutrients, boost immunity and protect against carcinogens.

- **CAMPYLOBACTER**
  - C. Jejuni and C. coli are the strains most commonly associated with human disease. Infection usually occurs through the ingestion of contaminated food.

- **ENTEROCOCCUS FAECALIS**
  - A common cause of post-surgical infections.

- **CLOSTRIDIUM DIFFICILE**
  - Most harmful following a course of antibiotics when it is able to proliferate.
Benefits of the Resident Flora

Structural

- makes up part of the intestinal barrier (mucosal)
Inflammatory Bowel Disease

An umbrella term covering all conditions which lead to chronic inflammation in the digestive system.
Can you use probiotics or prebiotics to treat IBD?

PREBIOTICS is a non-digestible part of foods (fibre usually or complex sugars) that travel through the small intestine undigested but broken down later by bacteria.

PROBIOTICS are live beneficial bacteria that are naturally created by the process of fermentation in foods like yogurt, sauerkraut, miso soup, kimchi, and others.

Lactobacillus – the most common probiotic found in yogurt and other fermented foods + help with diarrhea and may help with people who can’t digest milk sugar (lactose).

Bifidobacterium – May ease symptoms of irritable bowel syndrome (IBS) and related conditions. Naturally present, prevents constipation, breaks down carcinogens.
Mucosal-luminal interface proteomics reveals biomarkers of pediatric inflammatory bowel disease-associated colitis

Shelley A. Deeke MSc, Amanda E. Starr PhD, Zhibin Ning PhD, Sara Ahmadi MSc, Xu Zhang PhD, Janice Mayne PhD, Cheng-Kang Chiang PhD, Ruth Singleton RN, CCRP, Eric I. Benchimol MD, PhD, David R. Mack MD, Alain Stintzi PhD & Daniel Figeys PhD

The American Journal of Gastroenterology 113, 713–724 (2018)  Download Citation ↓
Home Grown Research

Gut-Brain Axis

Ottawa Researcher

Dr. Marie-Claude Audet
Inflammation and the microbiome: implications for depressive disorders

Shawn Hayley, Marie-Claude Audet, Hymie Anisman

https://doi.org/10.1016/j.coph.2016.06.001

Gut-Brain Axis

Ottawa Researcher

Dr. Marie-Claude Audet
Definition:
The **invasion** and sustained **colonization** of microorganisms into locations in the body where they are not expected to be.
Symbiosis

• Mutualism
  • Both members benefit from the interaction

• Commensalism
  • One organism benefits, and the other is neither harmed nor helped

• Parasitism
  • One organism benefits while the other is harmed or killed – most pathogens

• Amensalism
  • One organism can hamper or prevent the growth/survival of another without being affected by the other organism
Opportunistic Pathogens (Cont.)

• Compromised immune system
  • acute and chronic disease (primarily immune disease)
  • malnutrition
  • stress
  • age
  • radiation/chemotherapy
  • immunosuppressive drugs

• Changes in the normal flora
  • Normal flora is usually protective
  • Competition no longer exists

• Entrance of the normal flora into areas of the body where it is not present under normal conditions
Infection vs Disease

- Infection: an invasion and sustained colonization of an infectious agent in the host. The microorganism has to gain entry into the host and its tissue.

- Infectious Disease: when an infectious agent causes pathophysiological changes and disrupts normal physiological function.
Virulence and Pathogenicity

• **Virulence** is the degree of **pathogenicity** or disease-provoking power of a specific microbe

• Pathogenicity is related to the number of microorganisms, portal of entry, host defense, intrinsic characteristic of organism, and virulence factors

• Virulence is based on **virulence factors**
Virulence Factors

• **Virulence** is the degree of pathogenicity or disease-provoking power of a specific microbe and is based on virulence factors

• Virulence factors include the following
  • adhesion (specific or non specific)
  • colonization
  • invasion
  • evasion of host defenses
  • the production of toxins
Adhesion

• First and most crucial step of infection

• Without adhesion, organism will be removed by ciliary motion, sneezing, coughing, swallowing, urine flow, tears, intestinal peristalsis

• Bacteria must bind to host cell by pili or specific membrane receptor sites

• Adhesion can be nonspecific or specific
  • Nonspecific adhesion is through nonspecific attractive forces or interactions
  • Hydrophobic interactions, electrostatic attraction, atomic and molecular vibrations, recruitment and trapping by biofilms
Colonization

• The formation of a **compact population** by the infectious species

• Human pathogens usually colonize tissues that are in contact with the external environment
  • Urogenital tract
  • Digestive tract
  • Respiratory tract
  • Conjunctiva
Invasion

• The process of entering new areas of the body (breaking down barriers)

• May be aided by the production of extracellular substances
  • Disrupt host cell membrane
  • Break down primary and secondary barriers of the host
  • Are called *invasins*
Evasion of Host Defense

• Avoid contact with phagocytes (things that eat bacteria)

• Inhibition of phagocytic engulfment

• Survival inside the phagocytes

• Production of products that kill or damage phagocytes before or after ingestion
Portals of Entry (Cont.)

• Portals are generally the same areas that support normal flora: skin, GI tract, mucous membranes, placenta

• Majority of pathogens have their preferred portal of entry

• If pathogen enters the “wrong” portal, infection will not occur

• Some infectious agents enter via more than one portal
Portals of Entry (Cont.)

• Skin
  • Thick layer of keratinized dead cells
  • Pathogens can enter through natural openings, such as hair follicles and sweat glands
  • Damage of skin—abrasions, cuts, punctures, scrapes—these open the skin and allow microbes to enter
Mucous Membranes
GI Tract, Respiratory Tract, Urogenital Tract, Conjunctiva

• Gastrointestinal tract
  • Portal of entry for pathogens present in food, liquid, and other ingested substances
  • Enteric bacteria include: *Salmonella*, *Shigella*, *Vibrio*, and certain strains of *E. coli*
  • Viruses include the poliovirus, hepatitis A, echovirus, and rotavirus
  • Protozoans: *Entamoeba histolytica* and *Giardia lamblia*
  • Helminths: Trematodes, cestodes, nematodes
Mucous Membranes (Cont.)

• Respiratory tract
  • Most frequently used portal of entry
  • Pathogens enter through air, via dust particles, moisture, and respiratory droplets from infected people
  • Bacteria—causative agents of sore throat, meningitis, diphtheria, whooping cough
  • Viruses—agents causing the common cold, influenza, measles, mumps, rubella, and chickenpox
Mucous Membranes (Cont.)

• Urogenital tract
  • Pathogens usually contracted by sexual contact
  • Girls and women who are not sexually active are susceptible to lower urinary tract infections—close proximity of anus to urethra
  • Opportunistic infections by *E. coli*
  • Vaginal yeast infections—opportunistic by overgrowth of *Candida albicans*
Mucous Membranes (Cont.)

• Conjunctiva
  • the mucous membrane that covers the front of the eye and lines the inside of the eyelids
  • Usually good barrier against infectious agents
  • Some bacteria can easily attach to this membrane
Parenteral Route

• Technically not a portal of entry
• Portal of entry is evaded
• Pathogens are directly introduced to the subcutaneous tissue
  • Examples: Punctures by nail, thorn, contaminated needles
• Cuts, bites, stab wounds, deep abrasions, surgery
Toxins

• Organism that produces toxins is called *toxigenic*
  • Underlying mechanism by which microorganisms produce disease

• Bacterial toxins can be lipopolysaccharides associated with the gram-negative cell wall or proteins released from the bacterial cell
Exotoxins

• Protein released by bacterium
• During exponential growth phase
• Can act at sites other than the location of infection
• Toxins produced are often species specific and associated with a particular disease
Endotoxin

• Lipopolysaccharides of gram-negative cell wall (may also be embedded proteins)

• Release during lysis initiated by effective host defense or by action of antibiotics

• Can act on sites remote from the original site of infection

• Less potent and less specific than exotoxins
Actions of Exotoxins and Endotoxins
Examples of Exotoxins

The production of the toxin is generally specific to a particular bacterial species that produces the disease associated with the toxin (e.g. only *Clostridium tetani* produces tetanus toxin; only *Corynebacterium diphtheriae* produces the diphtheria toxin).

**Diptheria toxin**: Discovered 1871

A single protein (60kD) comprised of two peptide chains (A and B). Fragment B binds a receptor and helps the protein enter the cell. Fragment A inhibits protein synthesis by binding Elongation Factor 2 – EF2.

In 1901, Emil von Behring won the Nobel Prize in medicine for contributions to serum theory using Diptheria toxin.
Immunology

How the Body Protects Against Microbial Infection

Note: Immunology is Crazy!! We only have time to scratch the surface
IMMUNOLOGY

HERE'S A USEFUL DIAGRAM
Three Levels of Host Defense

First line of defense
Physical barriers
Skin
Mucous membranes
Tears
Saliva

Second line of defense
Inflammation response
Phagocytosis
Fever
Interferons
Complement system

Third line of defense
Specific immune response
Natural killer cells
Physical barriers

Skin

Mucous membranes, ciliary escalator

Perspiration, tears, saliva, flow of urine, peristalsis

Chemical barriers

pH: Skin, sebaceous and oil glands, mucus

Lysozymes present in perspiration, nasal secretions, saliva, and tears
Second Line of Defense

- Phagocytosis; ingestion and digestion
- Inflammation
- Fever
- Production of interferons/cytokines
- Activation of complement system
- Phagocytosis; ingestion and digestion
- Inflammation
- Pyrexia
- Production of interferons/cytokines
- Activation of complement system

- Effective inflammation prevents infectious disease by preventing invasion of adjacent tissues
- When injury occurs (i.e. cut in skin)
  - capillaries are damaged — release of bradykinin
- Stimulates mast cells and basophils to release histamine
  - Both bradykinin and histamine increase blood flow and capillary permeability
- Migration of neutrophils and monocytes to site of injury
  - Neutrophils phagocytose bacteria
  - Monocytes mature into macrophages when leaving the blood stream— phagocytose microbes
In response to a developing systemic infection, emergency myelopoiesis (left shift) rapidly produces both immature and mature neutrophils. This process is referred to as Neutrophilia.

Takizawa, Blood, 2013
• **Pyrexia** (Fever)
  • Systemic response to inflammation or microbial invasion
  • Makes bacteria/viruses more susceptible
  • Increases activity of some immune cells
  • Temperature regulation by hypothalamus in response to **pyrogens**
    • Released by white blood cells
    • Microbial toxins can also act as pyrogens

• **Production of interferons/Cytokines**
  • **Interferons**: Produced by cells infected with a virus. Interfere with replication of a virus and impede spread of pathogen
- Phagocytosis; ingestion and digestion
- Inflammation
- Pyrexia
- Production of interferons/cytokines
- Activation of complement system

• Activation of the complement system
  • Consists of more than 35 soluble proteins found in extracellular fluid
  • Activated by immune system in response to invasion
Third Line of Defense

• “Memory response”
• Specific or adaptive defense
• Triggered by specific antigen
• Cell-mediated and humoral immunity

-- End of Section on the Immune System --
Antibodies

• They are proteins—immunoglobulins (Ig)

• In circulation and specific receptors on B cells

• Produced by B-lymphocytes

• Recognize and bind to foreign antigens

• Form antigen-antibody complex
Epitopes: Antigenic Determinants

Bacterial Cell