HSS1100 Lecture 1-4

SUBJECT

- General Principles of microbiology
- Pathogenesis of Infectious Diseases
- Immunity to Infection
- Immunization (vaccination)
- Antibiotic Resistance

Quiz #1

- General principles of diagnostic microbiology
- Gram-positive cocci
- Gram-negative cocci

First Midterm Exam (20%)

- Gram-positive bacilli
- Gram-negative bacilli
- Mycobacteria
- Spirochetes
- Chlamydia, Mycoplasmas

Lecture 1: Microbiology and Immunology

General Principles of Microbiology

- micro = small
- bio = life
- logy = study (of) or science

- Immunology = study of our protection from foreign macromolecules or invading organisms and our responses to them

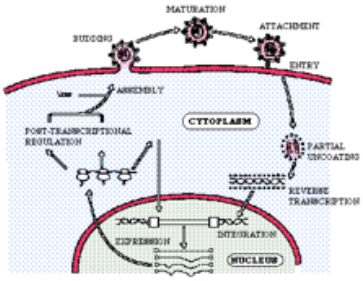
Different classes of organisms…

- Viruses / chlamydia (grow only in living cells)
- Mycoplasma (grow on non-living media)
- Bacteria (no separate nucleus; unicellular)
- Parasites

- Small (microscopic)
    - 1-2 microns (1 mm = 1000 microns)
    - Address them by their proper names !!!
    - Genus species: *Listeria monocytogenes, Listeria species, Listeria, Listeriosis*
        - (i.e., not “germs”, “bugs”)

What are they made of?

- Viruses
    - Nucleic acid (either RNA or DNA…never both!)
    - Surrounded by protein shell (capsid)
    - Attach, inject nucleic acid (penetration), highjack synthetic processes inside cells to make more viruses, package, get out while going is good…



- Bacteria
    - Rigid cell wall to keep things in place
    - Genetic material – circular chromosome
    - No nucleus (nucleoid)
    - Both DNA and RNA
    - Binary fission
    - Some bacteria do not have a rigid cell wall and are more fragile (i.e., Mycoplasmas)

- Eukaryotes
    - Unicellular and multicellular animals and plants
    - Genetic material is organized into a nucleus

- Are all bacteria bad?
    - biotechnology, spoilage of foods, bioremediation, functional foods, etc…
    - "Good" bacteria causes food spoilage that warns of hidden dangerous, harmful bacteria
- Can we live without bacteria?
    - "But as long as humans can't live without carbon, nitrogen, protection from disease and the ability to fully digest their food, they can't live without bacteria,"—Anne Maczulak, famous microbiologist

"Normal" flora…the good guys

- Resident versus Transient
- **Resident**: bacteria in gastrointestinal tract from birth, maintained throughout lives with population changes (from antibiotics, genetic diseases, etc)
- **Transient**: here today, gone tomorrow, can cause disease/symptom
- GI-tract: colon is inhabited by anaerobes and coliforms
- Skin: mostly coagulase negative staphylococci
- Where should there be NO bacteria?
    - Between open organs, tissue not exposed to outside, etc.
- Bacteria should be on: skin, digestive system, respiratory system (resident flora)

What can they do for us?

- protection from invasive (bad) bacteria
- metabolism (vitamin K), immune stimulation

What protects us from the bad guys?

- Mechanical barriers
    - skin, saliva, mucous, tears, hairs, etc.
- Other helpers include
    - antibodies
    - complement
    - immune cells (T-cells, NK cells, macrophages)
    - immune system (cell mediated; humoural)

How do the bad guys get in?

- Adherence
- Toxin production (destroys some of our defenses)
- Opportunism (usually no problems unless host is compromised or put in wrong location)
- Compromised host (how does this happen?: not 100%, not homeostasis)

- bacteraemia versus septicaemia? (aemia = blood)
    - **Bacteraemia**: presence of bacteria in the blood, form of septicaemia
    - **Septicaemia**: blood poisoning, includes lead, alcohol, etc. in blood

Infectious disease and the human (immune) response

- Infectious disease agents + human side, response (how, why, specific response)

Microbial disease

- Interaction between microorganisms and the host (us) is continuous battle
    - They need to enter-live-multiply
- In order to enter, they need to colonize (establish and multiply) in/on body; clinical infection (disease) can result when damage occurs to host [contamination = deposition without multiplication]
- Clinical disease = easy to recognize (symptoms)
- Sub-clinical infection = hard to diagnose (no symptoms)

Ways

- Colonize→ clinical infection OR
- Contamination (deposited without undergoing mechanisms to by-pass host defences)

How do we measure how dangerous a bacteria/virus/parasite is?

- Pathogenicity = ability to produce disease (can (or cannot) cause disease)
- Virulence = relative capacity to cause damage
    - (i.e., the degree of pathogenicity; how good the damage is - more=higher virulence)
- Opportunistic = do not normally cause disease but can do so when defense mechanism(s) breached or compromised

Pathogenesis of Infectious Diseases

- A pathogenic microorganism enters your body…two things happen:
    1. Microorganism (invader) tries to multiply / invade and cause disease (2º event)
    2. Host tries to prevent #1
    - Whether the invader wins or not is dependent on several factors

- Transmission:
    - inhalation, ingestion, break in protective barrier, direct deposit
    - Pathogenicity (ability to cause disease or not, cause disease in all or some, etc.)
    - invasiveness (adherence, persistence, avoidance of immune system)
    - toxigenicity (ability to make toxins)

How does a pathogen adhere to us?

- A bacteria needs to adhere, evade and invade the host
    - (different bacteria -viruses and parasites- use different strategies)
- Tools used to achieve these huge objectives:

    - surface structures (pili, fimbriae)
    - capsules
    - enzymes

Toxigenicity

- Toxins are substances (usually proteins) secreted by bacteria with the hope to cause damage
- Two classes:
    - Exotoxins (towards outside)
        - excreted by **living** cells
        - specific affinities (towards certain cells in the body)
        - Thermolabile (sensitive to hea)
        - potent
    - Endotoxins (inside the skin -cell wall- of bacteria, released when bacteria dies -cell wall disintegrates)
        - liberated when cell wall disintegrates
        - less specific, causes fever, malaise, shock
        - thermostable (no endotoxins in cosmetics or pharmaceuticals)
        - less potent than exotoxins

Lecture 2: What is Immunity?

- **Immunity** = the protection against infectious disease conferred either by the immune response generated by immunization or previous infection or by other nonimmunologic factors…a.k.a. body's ability to resist infection
- 2 types of immunity
    - Non-specific (innate)
        - Same reaction, occurs same way every time (1st line of defense)
    - Specific (adaptive, acquired)
        - Specific strategies from immune system

Innate Immunity

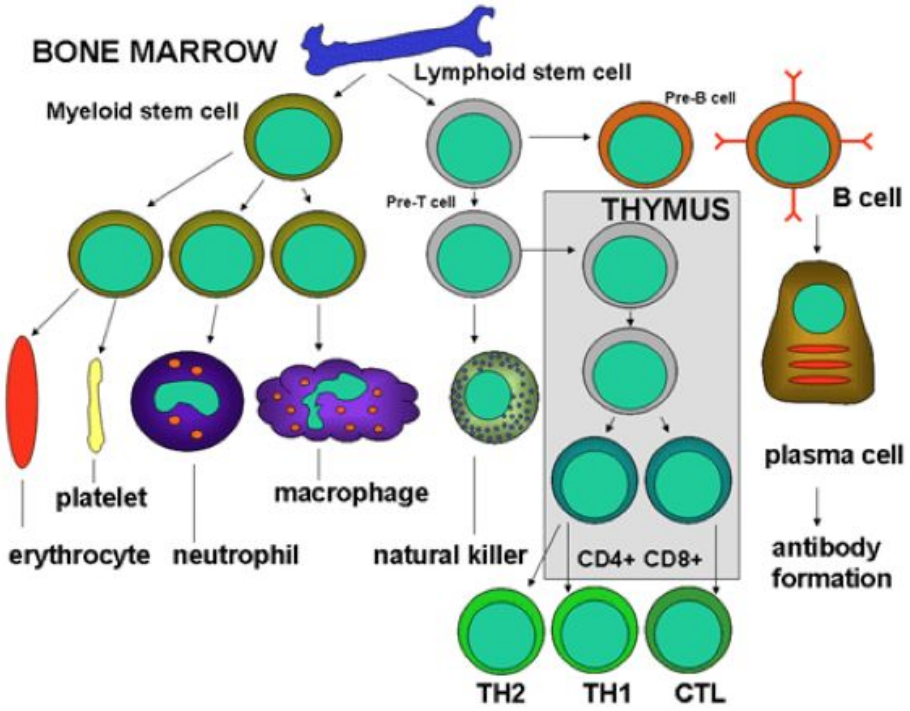
- **Skin**
    - What characteristics of the skin make it an effective mechanical barrier?
        - Skin is covered with layer off dead, keratinized epithelium that's too dry for bacteria to grow on and these cells are continuously sloughed off from the skin (with bacteria and pathogens still on)
        - Waterproof and pathogens can't enter unless compromised
- **Mucous membranes** (mechanical)
    - Cilia in respiratory tract
    - Lysozyomes, pH (e.g. stomach)
- **Iron-binding proteins** (bacteria cannot grow)
    - Some bacteria require iron for growth
    - Transferrin, lactoferrin (breastmilk)
- **Phagocytosis**
    - PMNs, monocytes and macrophages
    - (WBC -vacuum, good for cleaning variety of surfaces)
- **Complement**
    - Set of circulating proteins in blood
    - Part of immune system that enhances (complements) ability of antibodies and phagocytic cells to clear microbes and damaged cells, promote inflammation, and attack pathogen's membrane
    - Role 1: opsonization (immune process using opsonins to tag foreign pathogens for elimination by phagocytes)
        - Make antibodies to bond to more attractive (to immune system)
    - Role 2: MAC membrane attack complex; function accomplished through 3 broad effector pathways
        - Lysis (disintegration of cell by rupture of cell wall or membrane)
        - Inflammation
        - Opsonization/phagocytosi

Specific Immunity

- Humoral and Cell-Mediated (CMI)
- What is the difference between innate immunity and adaptive immunity?

- **Innate**: protects against ANY invader, does not discriminate (not trained response)
- **Adaptive**: directed against one type of invader, dependant on past exposure (i.e. why we vaccinate)

Where do immune cells come from?



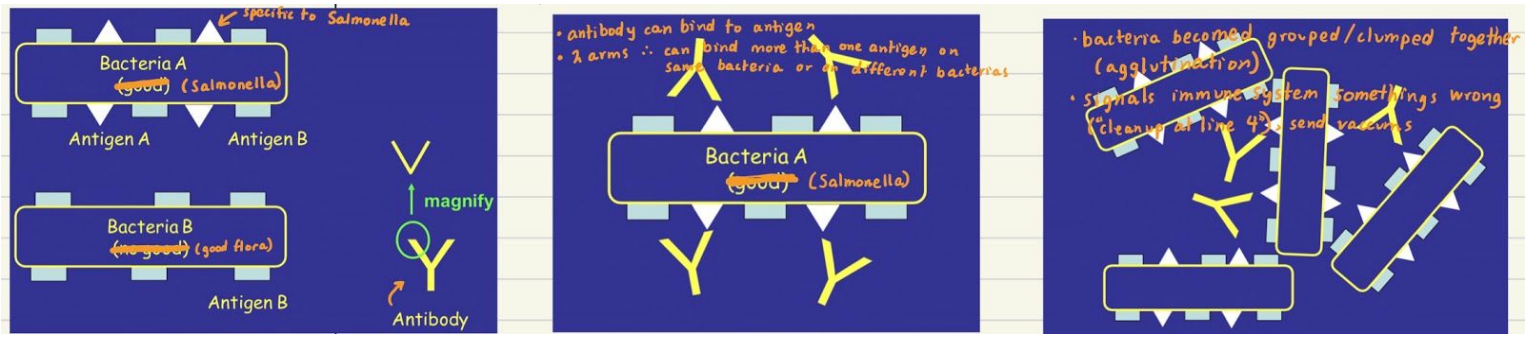
Two Specific Immune Responses

Humoral Immunity

- Circulating antibodies
- Antibody: protein that binds specifically to a substance (its antigen)
  - A.k.a. Igs or immunoglobulins
  - Produced by B-lymphocytes upon stimulation from antigen presenting T-cells
  - Recognize toxins, capsules, some viral proteins
- B-cells make antibodies
- T-helper 2 cells tell B-cells to make antibodies
  - B-cell (light), TH2 (light switch), TH1 (cell helper immunity)
- Antigen
  - "Non-self" (antibodies shouldn't recognize bodies own cell -autoimmune disease)
  - Protein, glycoprotein, lipoprotein, polysaccharide
  - What structures could be "antigenic" in a bacteria? Virus?
    - E.g. Influenza virus (major antigens on surface proteins)
    - E.g. bacteria (can vary pili -protein polymers- and M-proteins, surface lipoprotein VIsE)

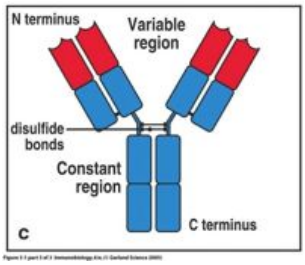
Antibody Binding: how does it occur?

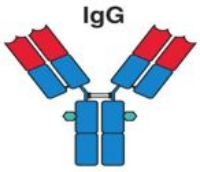
- Assume T-helper 2 cells has attached itself to a B-cell, telling ito make antibodies (Y - V)



Immunoglobulins (Igs) a.k.a. Antibodies

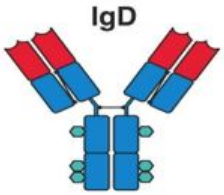
- Antibody: Ig produced in response to stimulation by an antigen and reacting <u>specifically</u> with it.
- Distinguish “non-self” from “self”
- Constant and variable region
    - **Variable**: responsible for antigen recognition
    - **Constant**: useful for allowing immune system to recognize it



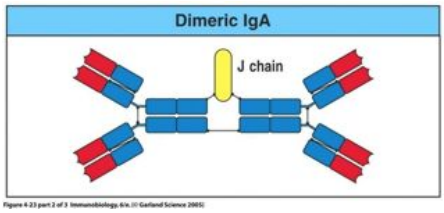


Classes of Igs

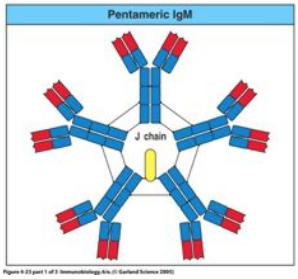
- 5 classes: IgG, IgA, IgM, IgE, IgD
- IgG
    - Host defense
    - Crosses placenta and protects newborn
- IgD
    - Role is unknown



- IgA
    - Host defense
    - Found in secretions
        - Tears, saliva, milk, respiratory, GI and genito-urinary tract
    - Dimer



- IgM
    - Host defense



    - Early immune response (first antigen introduction)
    - Pentamer

- IgE
    - Hypersensitivity (allergies)
    - Defends against parasites

1º and 2º Immune Response

- B-cells to be told by T-cells to make antibodies 2 ways
- 1º Response
    - Ab production triggered on first antigen introduction
    - Latent period of several days
    - Circulating antibody detectable after 5-10 days
    - Antibody in serum is maximum at ~21 days, then drops to low levels
- 2º Response
    - Basis for Immunizations
    - Occurs when Ab is introduced 2nd, 3rd,4th …time
    - Lag, rapid Ab increase (2-3 days), slow decrease
    - Booster injections to maximize Ab levels

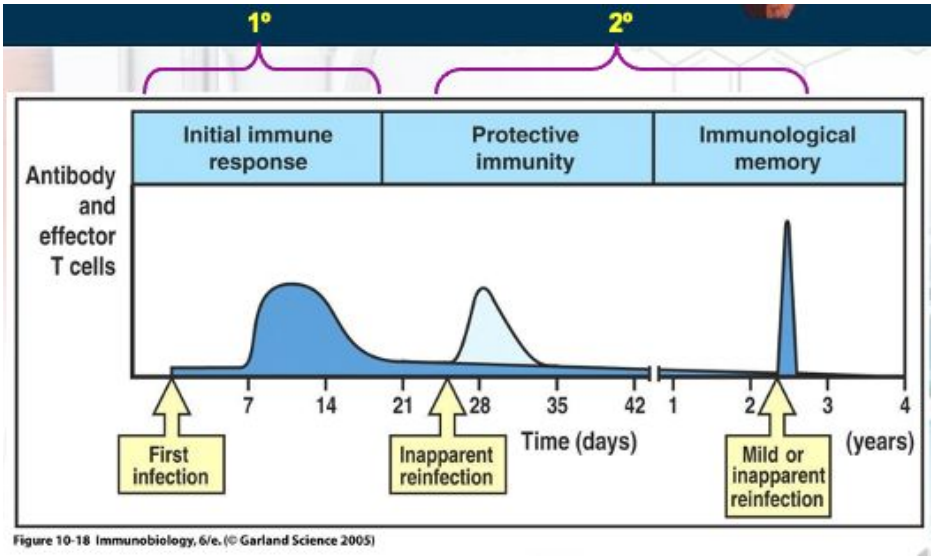
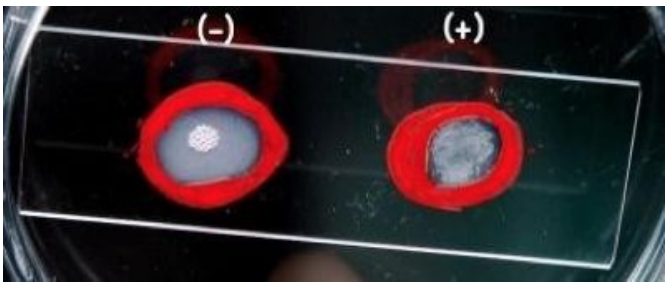


Figure 10-18 Immunobiology, 6/e. (© Garland Science 2005)

Antibody Detection

- Serological Reaction
    - Detects presence of antibodies in serum sample
    - Antigen and antibody interact; agglutination
    - Antibody titration
    - Detect unknown microorganisms using known antisera

Look for agglutination



Cell-Mediated Immunity (CMI)

- Used if antibodies doesn't help get rid of pathogens OR pathogen hides inside cells (intracellular)

- T-cells NOT antibodies!
    - Helper, suppressive, cytotoxic (killer) generated from memory T- cells
- Exposure to antigen induces response from trained T- cells
- Essential for defense against intracellular organisms, parasites, tumors and other foreign cells (i.e., transplants, grafts)
- Immune-suppressive medication for transplant recipients

How does your immune system know how to process an antigen?

- How does your body know whether to activate humoral or cell-mediated immunity based on the antigen it sees?
    - **Humoral:** T-helper 2 (find B-cells , make antibodies)
    - **Cell-mediated**: T-helper 1 response
    - Answer: Antigen-Presenting Cells are your best friend

General role of the Antigen Presenting Cell (APC):

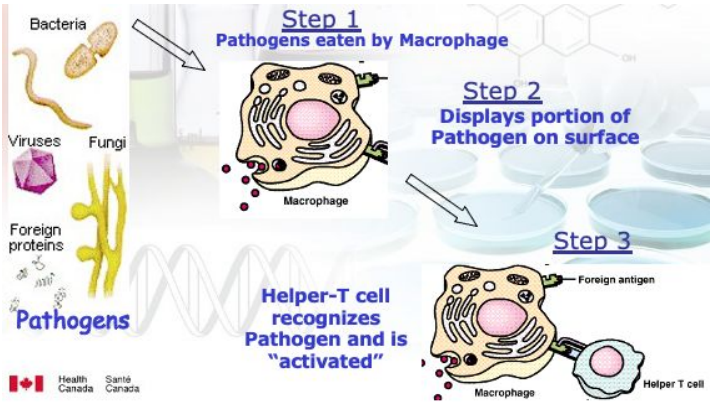
- Take in the « entity » and prepares antigen for presentation
- Presentation depends on how antigen is viewed
    - Intracellular versus extracellular
- Antigen presented on surface of APC and binds special receptor on T-helper cells
    - Chemicals (interleukin, etc.) help direct the response
    - Receptors are either MHCI (TH1) or MHCII (TH2) (major histocompatibility complex)
    - T-helper cells are Th1 or Th2

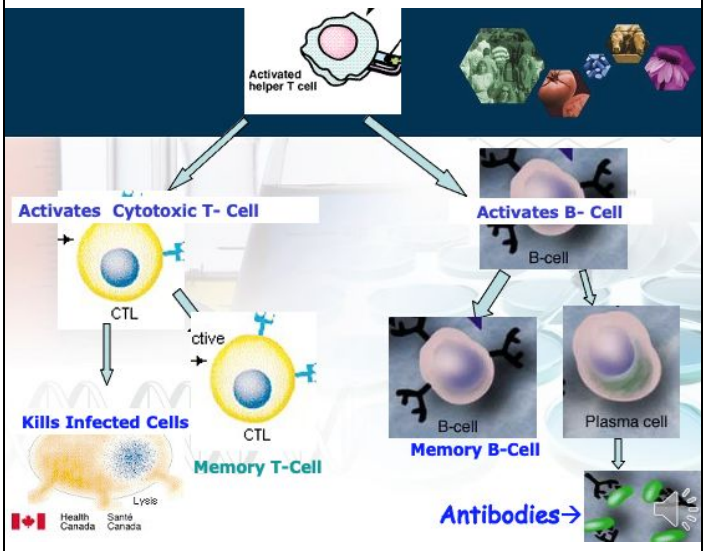
1. **T-helper 1**: mediates cell-mediated immune response
2. **T-helper 2**: attaches to B-cells, tell it to produce one of antibodies
3. **Antigen presenting cell**: ties it together, takes in antigen, processes and presents to TH1 or TH2

Pathway of Specific Immune Response

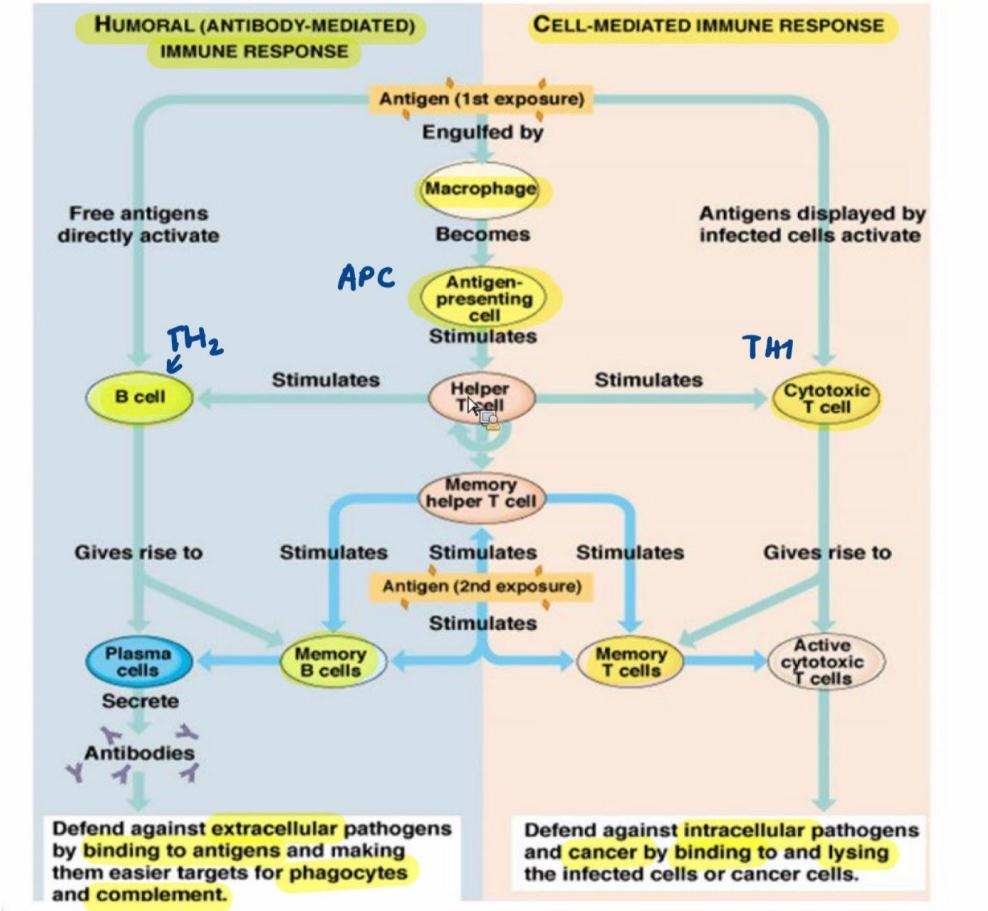
Step 1:
Takes in and Processes

Step 2:
Shown to TH1 or TH1



TH1 **Memory T-cell: Secondary Immune Response**		**TH2 → find B-cells** **Memory B-cell: for future**

Overview of the Immune Response



Disorders of Immunity

1. Allergy and Hypersensitivity
    - OVER-reaction to antigens in absence of true infection
    - Can be fatal…..ANAPHYLAXIS
2. Auto-immune diseases
    - Immune system reacts to its own “self” antigens
    - “auto-antibodies”
    - Type I diabetes, MS, rheumatoid arthritis, lupus
3. Immunodeficiency states
    - Inability to produce antibodies and/or disfunctional CMI
    - Congenital, disease, AIDS
4. Graft rejection
    - NORMAL immune reaction to “non-self”
    - Control by immune-suppressive medication

Immunization

Passive Immunization

- E.g. useful when going to foreign country; breastfeeding creates pre-formed antibodies from the mother
- administration of pre-formed antibody against a specific microbial agent
- **IgG animal origin**: short lived, risk of hypersensitivity reaction
- **IgG human origin**: short lived, no risk of reaction
- Gamma globulin (IgG): pooled from large grouped of blood donors and has antibodies to many common infections
- Hyperimmune globulins (IgG): specific for a particular microbe

Active Immunization (preference)

- Stimulates immune system by administration of antigen
- LONGER LASTING (memory cells formed; e.g. not salmonella but the specific salmonella antigens)
- Live-attenuated vaccine
- Sub-clinical or mild illness mimicking the disease
- Local (IgA) and humoral (IgG) immunity
- Rapid immunity development
- Serious illness in immuno-compromised individuals

- Killed vaccines, sub-unit vaccines and toxoids
    - Antigens without infectivity
    - May require boosters
    - Adjuvant with toxoids
    - Polysaccharide vaccines can be conjugated to protein (see conjugate vaccines)
- Recombinant vaccines
    - (insert DNA encoding an antigen that stimulates immune response into bacterial or mammalian cells)

    - DNA recombinant technology
    - Attenuates microorganism
    - Hep B vaccine
- **Ad**sorbed vaccines
    - Vaccine mixed with inorganic salt for slower adsorption and longer-lasting immunity
    - Tetanus, diptheria
- Conjugate vaccines
    - Designed for poorly antigenic microorganisms
    - Conjugate antigen of interest to immunogenic, non-toxic protein
    - Haemophilus influenzae type b
- Combined vaccines
    - For ease of administration
- Combined Active-Passive Immunization
    - Immediate protection after possible exposure to microbe
    - Hyperimmune Igs and vaccine injected at DIFFERENT sites
    - Tetanus, Rabies, Hep B

Canada's Immunization Guide

- Based on high risk pathogens that don't allow primary immune response
- https://www.canada.ca/en/public-health/services/canadian-immunization-guide.html

Antibiotic Resistance

Introduction

- The first antibiotic (?) PENICILLIN
    - discovered in 1929 by Sir Alexander Fleming
- World War II
    - penicillin used to treat staphylococci and streptococci (1946)
- How effective was penicillin?
    - So effective that it was thought to be the cure for all infectious disease. Thought they won the war against pathogens
- Resistance to penicillin recognized almost immediately
    - 80% of all strains of Staphylococcus aureus have resistance
    - Streptococcus pyogenes (Group A strep) still treated with penicillin
    - Interestingly, penicillin has never been effective against Gm-negatives (Salmonella, Shigella, Bordetella pertussis, Yersinia pestis, Pseudomonas) – why?
        - It still works on Gram-negative *Neisseria gonorrhoeae*.
        - Gram-negative bacteria are inherently resistant because their vulnerable cell wall is protected by an outer membrane that prevents permeation of the penicillin molecule.
        - have a lipopolysaccharide and protein layer that surrounds the peptidoglygan layer of the cell wall, preventing penicillin from attacking.
- Late 1940s and early 1950s?

- Molds, other organisms with antimicrobial effectiveness

Antibiotic therapy

- Effective chemotherapy depends on selective toxicity
  - good against pathogens, does not affect the host… (target something unique to pathogen)
- Exploit pathogen processes not seen in humans
  - cell wall, metabolism, etc.
- Knowledge of likely microorganism is crucial…
  - Site (where? Determines type of therapy: oral, injection, etc.?)
  - Organism (difficult since similar symptoms)
  - allergy to host?
- Other considerations…
  - route of administration (oral, intravenous, topical, etc.)
- Monitoring therapy (how to know if it's working)
- Adverse effects (negative effects on:)
  - GI-tract, skin, haemopoietic system, renal system, liver

Acquired resistance

- Three major mechanisms of resistance
  - Alteration in drug target
    - If antibiotics finds square molecule unique to pathogen; pathogen can change square into triangle and antibiotic can't bind anymore
  - Production of inactivating enzymes
    - Some bacteria can create & secrete things that can chop up and inactivate the antibiotic
  - Decreased uptake of antibiotic
    - 1. Make cell wall less permeable so antibiotics can't get inside
    - 2. Bacteria produces efflux proteins (proteins that bind antibiotics and throw it to outside as quickly as it entered)

Antibiotic resistance

- Resistance occurs when a susceptible microorganism is no longer inhibited by an antibiotic agent
- Many reasons why this can happen
  - intrinsic - characteristics of microorganism vis-à-vis antibiotic's mecanism of action (inherent or "natural")
  - acquired - new or added (driven by two genetic processes in bacteria…mutation and selection (vertical evolution); and exchange of genetic material (horizontal evolution)

The chromosome: role in antibiotic resistance…

- Mutations lead to
  - Change in site of antibiotic target (but protein for bacteria still works fine!)

    - Regulatory genes
        - turn on alternative path
        - turn on efflux mechanisms
    - Change cell permeability

Post-antibiotic era: is it possible?

- With current overuse of antibiotics, we are forcing bacteria to change (evolve) in order to survive
- How is this achieved/helped by us?
    - Overuse, broadspectrum antibiotics (kill a lot of things; incorrect approach)

Decreasing antimicrobial resistance?

- Withhold antibiotics
    - self-limited viral infections (i.e., the “common cold”)
- Use narrowest spectrum antimicrobial agents
- Base decision about broadness of empiric antibiotic coverage on
- severity of illness
    - clinically stable and not at risk for significant morbidity…may be appropriate to wait culture results and MIC testing
- Prevention of infection
    - hygiene, handwashing
- Education
    - helps to achieve therapeutic and preventative goals
    - When are antibiotics needed?
    - how to take them?
    - proper duration!!
- Earlier detection of therapeutic failure
    - good for patients with antibiotic-resistant pathogens

Why not make new antibiotics?

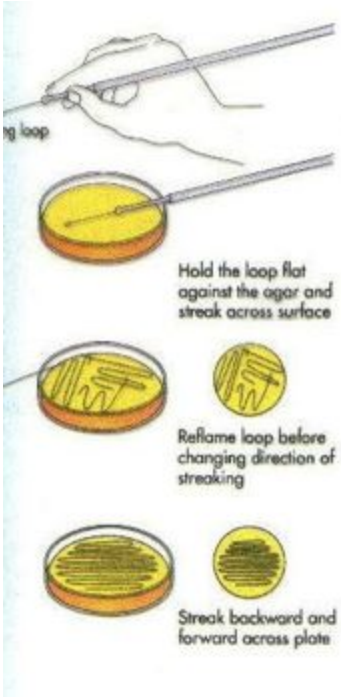
New antibiotics are seen as 'drugs of last resort' against dangerous bacteria. So, to limit the development of antibiotic resistance, they need to be used sparingly – and not sold in large volumes. Plus, compared to more expensive treatments, antibiotics tend to be quite low in price.

- **A “no-self” substance that can provoke an immune response?** ANTIBODY
- **Active artificially acquired immunity results in . . .** VACCINATION
- **A type of disease that results from the inability of the immune system to distinguish self from non-self antigens.** AUTOIMMUNE DISEASE
- **Cells that stimulate both arms of immune response.** HELPER T-CELLS
- **Without** LYMPHOCYTES**, there is no adaptive immune system.**
- **Innate immunity is present since birth.** TRUE
- **Immune response to non-self antigens or self antigens.** OTHER

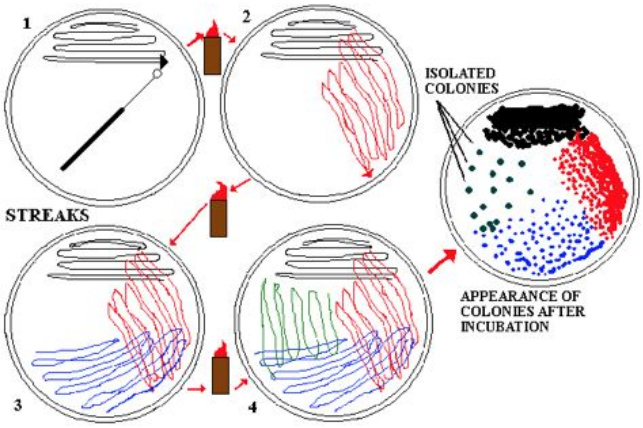
Lecture 3: General Principles of Diagnostic Microbiology

Diagnostic Microbiology

- Sick patient with blood sample, salmonella
- Isolation of pure culture from specimen
    - Why isolate and not just treat it? Origin, Specific stran, Directed treatment, mutation
- Culture media
    - Why take cerebrospinal fluid from a patient?
    - Why use a specific media to isolate pathogens?
    - What is media chosen?
    - Who are players (pathogens causing problems)?
- Inoculation methods
    - Clinical specimen (blood, urine, CSA, pus, food)
    - Ways to inoculate media
    - Streak (preferred, on top of agar), spread, or pour (embedded inside agar)



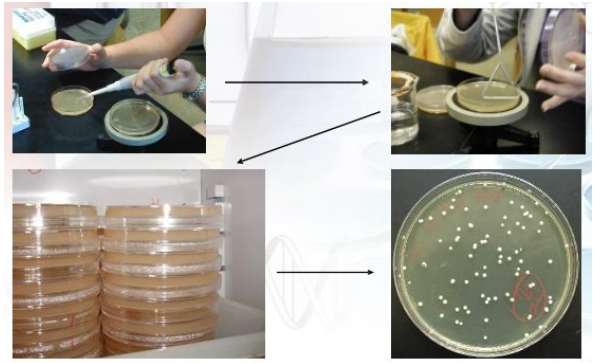
Streaking a plate for isolated colonies



Pour plates

- Bacteria grow inside agar
- Used for enumeration of bacteria
    - Put CSF sample + liquid sample
    - Incubate and see bateria (hopefully)
- Issues with cross-contamination

Spread plates



- Like streak plate, use media that's already prepared
- Dilute blood in saline, plate an amount or alowate and spread evenly with hockey stick, put in incubator
- Next day, dilution able to see amount of colonises (how many colonies per mL of blood)
- **Streak**: isolate pathogens, **pour**: isolate, **spread**: count how many we have (quantify)
    - CSF, make dilutions and spread
    - Eventually, a plate with countable bacteria

Preservation of Cultures

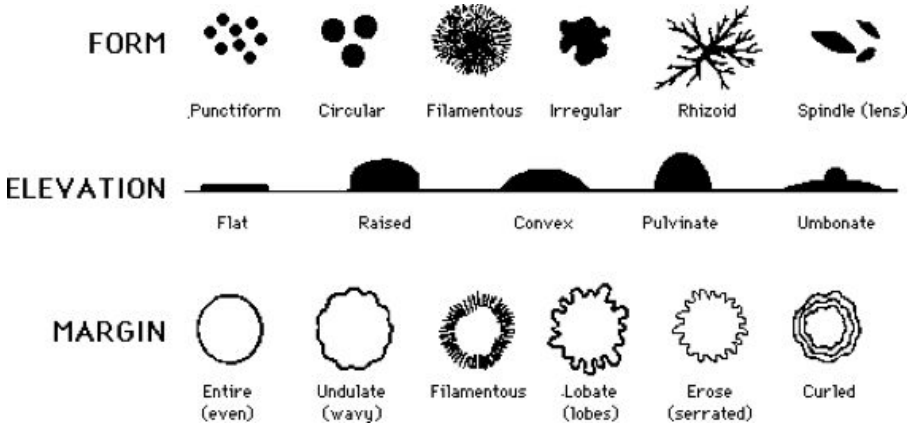
- pure cultures of bacteria are stored:
- freeze-dried
- (lyophilized)
- frozen at -80C- -120C

- Why would we want to keep a “copy” of a bacteria we isolated from a patient?
- Short term versus long-term
    - liquid nitrogen (-195oC)
    - freezers
    - lyophilization (freeze-drying)

Identification

- Now that you have a pure culture…
    - **colony** morphology
    - **cellular** morphology
- The microscope is your friend
    - **resolving power (resolution)** = ability to distinguish two closely located objects as separate, distinct entities
        - Closer two things are together, higher resolving power (see separate things as separate)

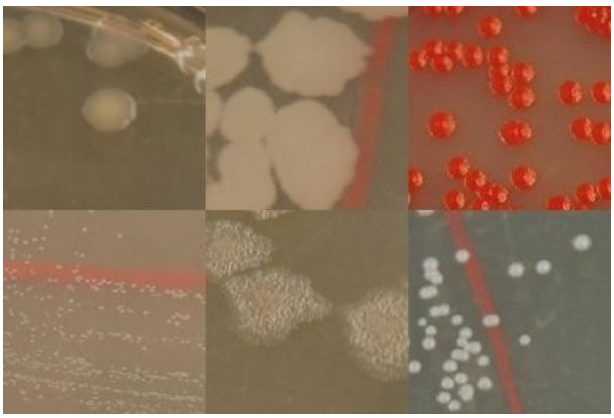
Colonial morphology



Margin: zoomed into colonizes

What you see gives you a good idea of what it is

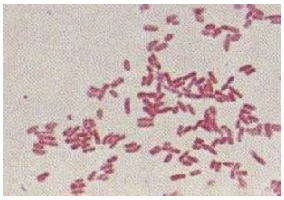
Examples of colonial morphologies

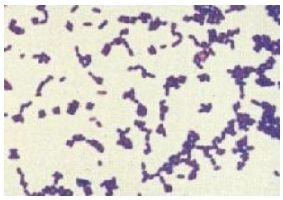


Different shapes and colours, same magnification

- Staining Techniques -

- **Colony**: one single bacteria touches agar and grows and divides to make a million - billion identical copies of itself
- Generally, three steps:
    1. Make a smear (saline on glass slide)
    2. Fix dried smear by heat (pass by fire quickly → feature a little) fixatioon
    3. Stain with desired dye

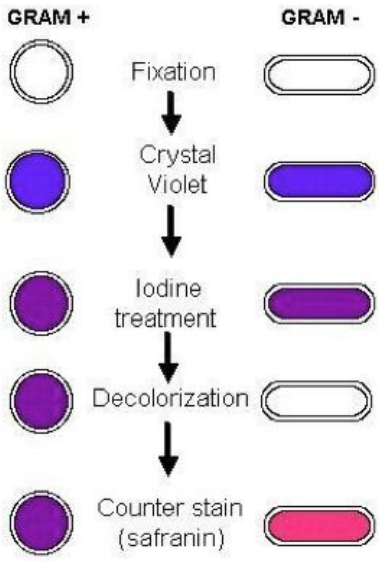




Simple vs. Differential staining

- Simple stain (problem with patient, take sample of blood or CSF, heat fix, add dye
    - Stuff seen: useful
    - Nothing happens: think of toxin or virus
    - single dye normally used
    - all organisms same colour
    - size, shape, number, arrangement, etc.
- Differential stain (done with isolated)
    - two or more dyes
    - differences between classes of microorganisms or parts of cells
    - acid fast, **Gram**

The Gram Stain (Hans Christian Gram)



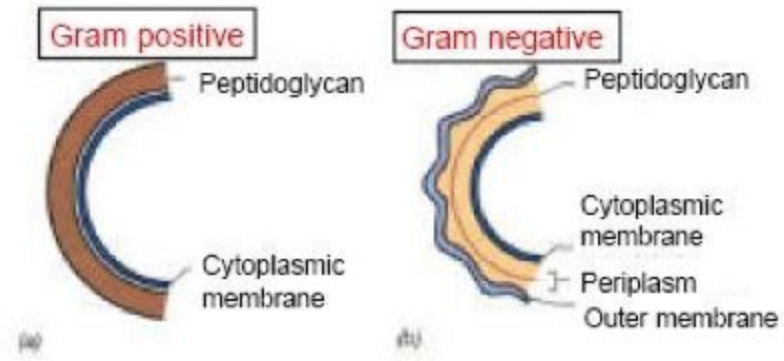
1. Flood slide with crystal (or gentian) violet. (Wash with running tap water).
2. Flood with Gram's iodine. (Wash with water). (both attach to something really well)
3. Carefully decolorize with 95% ethanol. (Wash with water).
    a. Ethanol cause Gram - to lose purple and become colourless, Grame + remain purple
4. This third step is the most critical and also the one most affected by technical variations in *timing and reagents.*
5. Flood with safranin (pink color). (Wash with water). Air dry, or blot with absorbent paper
    a. Safranin makes Gram - to be pink, Gram + takes in dye but still stays purple

- Certain antibiotics for gram + and totally different set of antibiotics for gram -

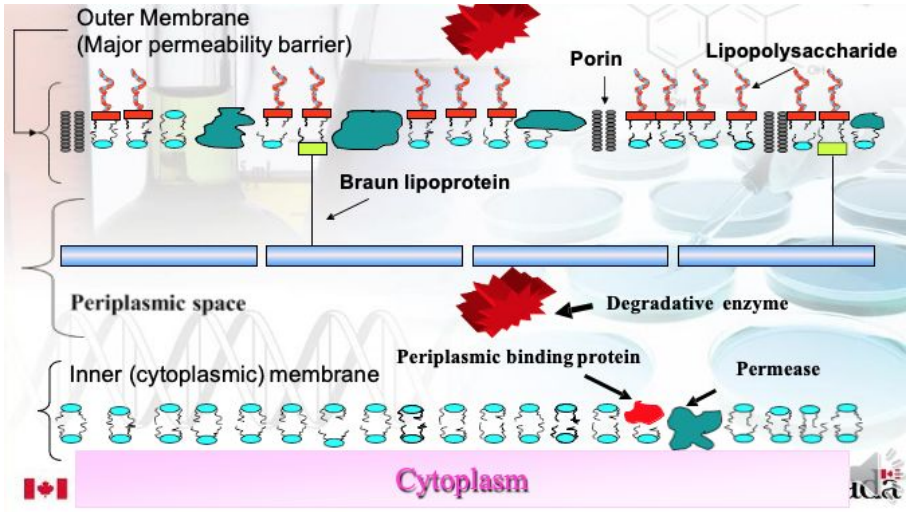
Cell wall is the key!

- Essential for cell growth and division

- Shape of bacteria related to peptidoglycan layer (inside cell wall of BOTH Gram - and Gram +)
- Gram negative usually thinner than Gram positive



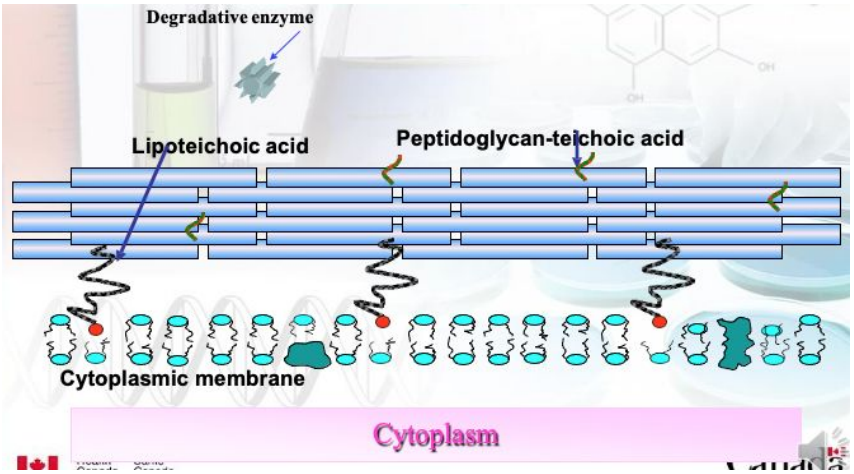
GRAM NEGATIVE CELL ENVELOPE



endotoxin released

- Cell wall → space → 2nd cell wall or outer membrane
- In periplasmic space, thin layer of peptidoglycan
- When doing Gram stain, **crystal violet** is added which binds here
- **Iodine** is added and also binds here and hangs onto peptidoglycan layer
- 3. **Ethanol**, when timed, will solubilize everything (outer layer + peptidoglycan layer) are washed away
- Cells no longer purple; colourless
- **Safranin**, bind here and make gram negative pink

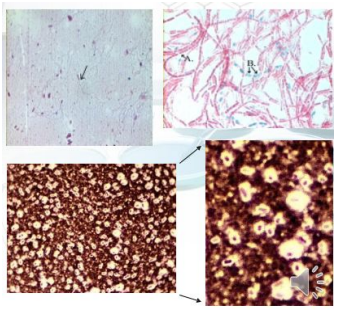
GRAM POSITIVE CELL ENVELOPE



- Inside of bacteria, cell wall, 2 differences: no secondary cell wall, really thick peptidoglycan layer
- Gram stain: crystal violet, iodine, **ethanol is timed state (messing up causes wrong diagnostic)**: some of peptidoglycan is washed (thick layer so only some layers wash away). Bottom layers remain
- Safranin binds here but still stays purple from crystal violet and ethanol

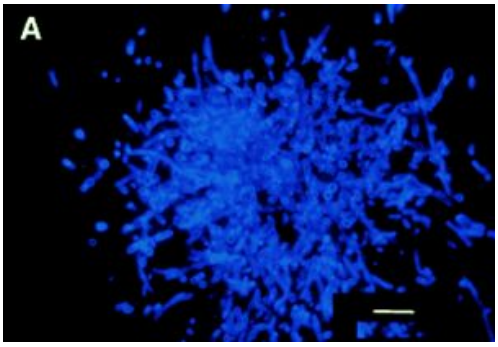
Other stains

- Endospore
- Capsule
- Flagella



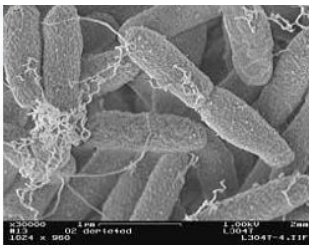
Fluorescence microscopy

- dye fluoresces at specific wavelength
- antibodies tagged with dyes are common (immunofluorescence microscopy)



A section of the liver of a leukemic patient who had succumbed to culture-proven C. *albicans mycosis.* (staining done with Blankophor)

Electron microscopy



- Electron beam (instead of light)
- Million times magnification possible (0.003 µm)
    - TEM (stain with heavy metals)
        - Transmission electron microscope
        - Open or cut individual bacteria to see
    - SEM (3-D image of cell surface)
        - Scanning electron microscope
        - Electron beam (not light) gives 3D picture of bacteria (no more slide)

Electron microscopy

Scanning electron microscope (SEM)

Transmission electron microscope (TEM)

Health Canada Santé Canada

So what's the bottom line?

- Clinical specimen, isolate colonies:
- Morphology helps to classify and identify
- Specific or differential stain
    - Gram stain

- Other stains:
- Gives clues to how they behave in environment (how patient got sick in first place)
    - capsules, endospores

Characteristics of bacteria

- Small (0.75 – 1.25 µm in diameter/width)
- Higher surface area / volume ratio
    - higher metabolism
    - faster growth
    - replication rate (~20 minutes)

Shapes and sizes of bacteria

- Bacteria are usually arranged in specific patterns:
    - single cells (spiral and/or rod shaped)
    - diplococci (pairs) – single plane
    - chain (divide in one plane and remain attached)
    - tetrads (cocci dividing at right angle to first plane of division)
    - division in three planes (grapelike clusters)
    - cubical packet of 8 cells (sarcinae)

Shapes and sizes of bacteria

- Clues to identify what is making patient unwell

With what do we grow bacteria? AGAR

 **Salmonella typhimurium** **Gram Negative:** growth **Lactose Fermentation:** negative (colorless colonies)	 **Escherichia coli** **Gram Negative:** growth **Lactose Fermentation:** positive (pink colonies)

Definitions for food used to grow pathogen

- **Chemically defined** – exact composition known (e.g. agar used to grow salmonella, control all components -dyes, glucose, etc.)
- **Chemically undefined** – some components can't be controlled (beef extract, blood, etc.) (e.g. blood agar -has red blood cells but varying amounts)

- **If solid (versus liquid) growth** – 1.5% agar used
- **Enrichment media** – increase # of specific bacteria in sample by favouring growth of interested species
- **Tissue culture media** – for cultivating viruses, derived of plant or animal cells (viruses need living media, tissue culture)

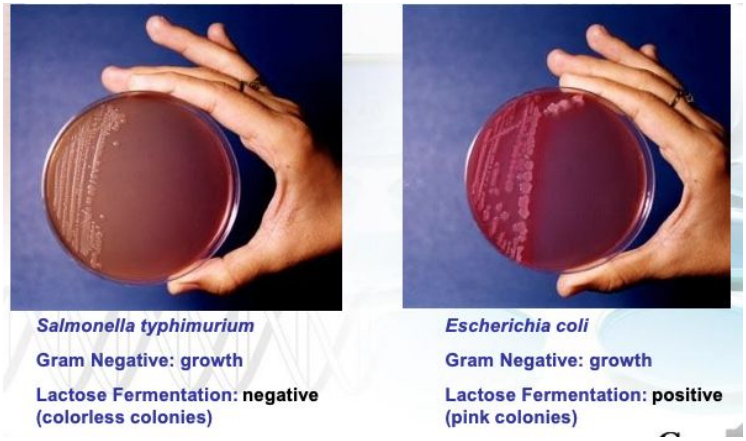
General media requirements

 **Bacteria** – requirements vary	 **Yeasts** – high sugar and lower pH	 **Anaerobes** – must remove oxygen **Anaerobic infection:** remove oxygen or else bacteria will die

Agar media: Selective, differential and S/D media

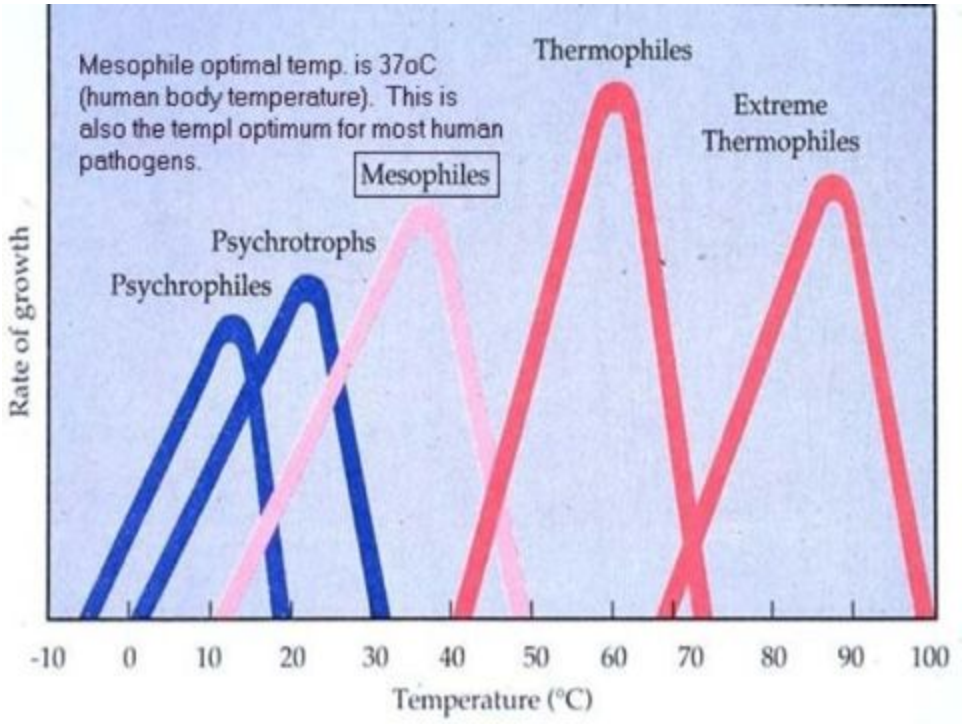
- **Selective media** – enhance growth of one bacterial species or suppression of another
    - E.g. Encourage growth of gram +, suppress growth of gram -
- **Differential media** – differentiate bacteria based on their nutritional requirements and phenotypic characteristics
- **Selective / Differential media** – very useful in clinical labs (e.g., MacConkey agar)
    - Most agars are selective and differential: agar plate for gram +, gram - are suppressed. Media has differential components to tell gram + apart

MacConkey Agar – S/D media Gram Negatives
(Bile salts, crystal violet inhibit Gram +ves; neutral red dye helps detect lactose fermentation)



- Things inside to let Gram -ve to grow but contains bile salt and crystal violet that inhibit Gram +ves.

Temperature requirements



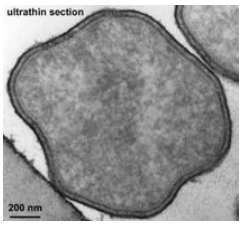
- Different bacteria have different food and temperature requirements
- Most infections caused by **Mesophiles**: optimal - 35 -37C = human body temperatures
- **Thermophiles** or **extreme thermophiles**: not a problem for clinical infection
- **Psychrophiles, psychrotrophs**: lower temp. = listeria (grow and survive refrigeration

Temperature definitions

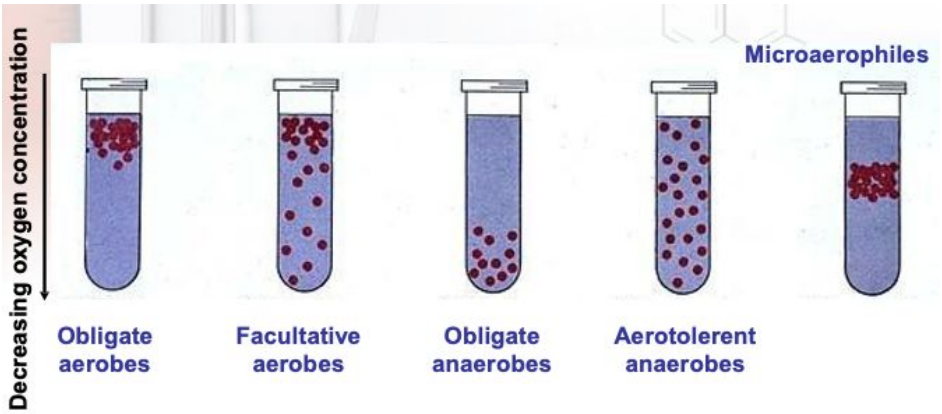
- Psychrophiles (lower temp)
    - grow best at temperatures 15-20oC
- Mesophiles (human temp)
    - grow best at temperatures 25-40oC
    - most bacteria belong here
    - Why fevers cause increase in temperature
- Thermophiles (higher temp)
    - grow best at temperatures 40-85oC

Extreme Thermophiles

- Pyrolobus fumarii
- “fire lobe of the chimney”
- Lobed shape
- Discovered in the walls of a deep sea hydrothermal vent
- Grows between 30 and 113C
    - 106C is optimal



Oxygen requirements



- Like food and temperature, oxygen requirements
- **Obligate aerobes**: need oxygen
- **Facultative aerobes**: does well in both but likes oxygen
- **Aerotolerant anaerobes**: does well in both but likes no oxygen
- **Obligate anaerobes:** opposite of obligate aerobes, no oxygen
- **Microaerophiles**: specific bacteria; need specific amount of oxygen, too much or too little kills them

Growth of anaerobic bacteria

Anaerobic jar

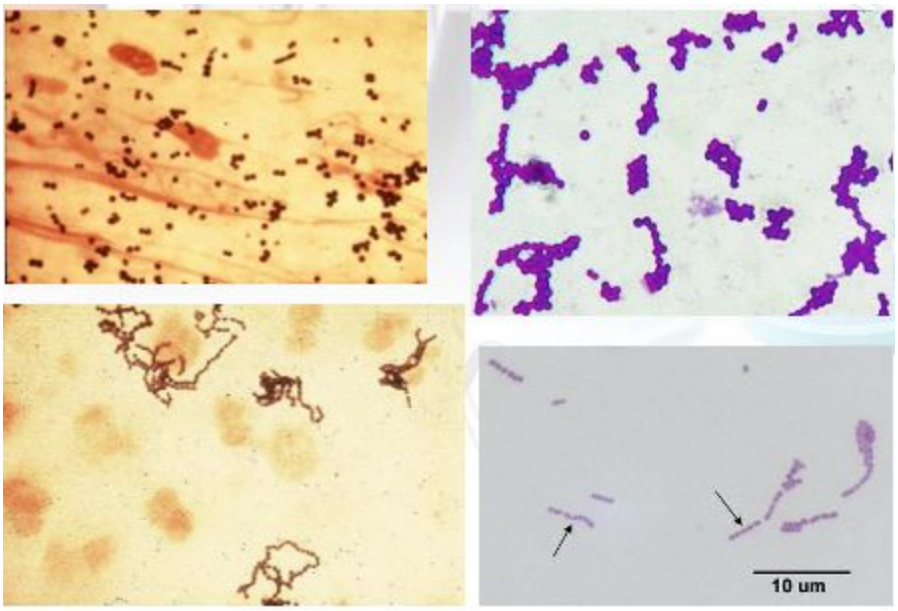

Coy anaerobe chamber

- E.g. claustritium, anaerobic, blood specimen, urine, CSF: no oxygen present
- Anaerobic jar/chamber, petridish, gas pack, water: soaks up oxygen. Indicator strip tells if oxygen is present

pH and Water requirements

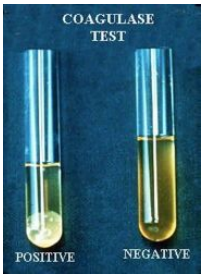
- Optimal pH varies from bacteria to bacteria
- Intracellular pH must be ~7.5
- Growth observed at pH values of 4-9 (optimum 6-8)
- Water (light) can be important for certain microorganisms
- Osmotic pressure (hypertonic, hypotonic, isotonic) (blood, CSF put in liquid media not solid media like usual)

Lecture 4: Gram-positive Cocci & Gram-negative Cocci
Gram Positive Cocci



Staphylococcus aureus

- "*Staphule*" means grape in Greek
- Toxins are quite the problem:
    - Cytotoxins
    - Haemolysins (destroy red blood cells)
    - Enterotoxin (A-E, G-I)
        - Exotoxin affecting gastrointestinal track
    - Exfoliative toxins (ETA, ETB)
        - Causes scalded skin symptoms
    - Toxic shock syndrome toxin 1 (used to be exotoxin C and enterotoxin F)
- Enzymes:
    - Coagulase (coagulation of fibrin) (clumping)
        - made by almost all pathogenic staphylococci
        - used in laboratory test to differentiate from *S. epidermidis, S. capitis and S. saprophyticus*
    - Beta-lactamase (penicillinase)
        - destroys penicillin



- Many *S. aureus* strains are found in normal population (~15%)
- Carried in anterior nares, axilla, perineum and hands
- Problem:
    - 85-90% of strains isolated in hospital are penicillin resistant!!!
    - Localized purulent infections (pustules, boils, styes, conjunctivitis, otitis, etc.)
    - Pneumonia, osteomyelitis, septicaemia, endocarditis
    - Food poisoning, toxic shock syndrome, scalded skin syndrome
- Important cause of hospital acquired nosocomial infections from stitch abscesses, infected wounds, or generalized infections

- Preventative measures include
    - Aseptic technique in ER and OR, wound precaution
    - Education of health personnel
    - Handwashing!

Staphylococcus epidermidis

- Part of normal skin/mucous membrane flora
- Non-pathogenic, except in compromised patients where can cause post-operative infections (brain, open heart, endocarditis, shunt infections)
- Considered an opportunistic pathogen

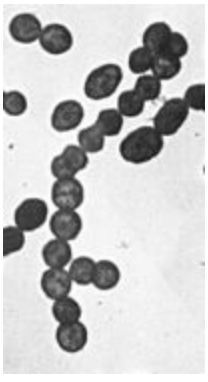
Streptococci

- Arranged in pairs or forming chains
- “streptos” - Greek word for twisted
- subdivided into “groups” based on
    - haemolytic properties (alpha, beta)
    - carbohydrate C antigen (Lancefield classification)
    - M-protein
        - divides beta-haemolytic
        - mostly group A

Rebecca Craighill Lancefield (1895-1981)

Streptococcus pyogenes

- Group A, beta-hemolytic, S. pyogenes causes:
    - acute tonsillitis (strept throat) – can lead to rheumatic heart disease
    - impetigo, cellulitis, etc. (skin infections)
    - fever and septicaemia
- Caused by toxins
    - streptolysins (O and S)
        - neutrophils and macrophages
    - streptococcal pyrogenic exotoxins (Spes)
        - scarlet fever rash



Streptococcus pyogenes

- Enzymes
    - hyaluronidase (helps spreading of bacteria)

- Virtually all are penicillin G sensitive (vs. *S. aureus*)!!!
- Education of health personnel
- Aseptic obstetric procedures
- Early detection and treatment

Flesh-eating disease … aka Necrotising fasciitis

- Streptococcus pyogenes culprit
- Does not actually "eat" anything
- Toxin is responsible for damage
- Research indicates that
    - hijacking human plasminogen from blood, attach to surface and activate it into protease…good for spreading…
    - bacteriophage has gene encoding for enzyme allowing bacteria to escape entrapment and killing by neutrophils (white blood cells)

Streptococcus agalactiae

- Group B
- Found in vagina of health women (can cause neonatal infections)
    - early septicaemia
        - respiratory distress or shock at birth
        - high fatality rate (serious)
        - delayed meningitic form
    - 1-12 weeks post-partum
        - sequelae

Other Streptococci

Streptococcus faecalis

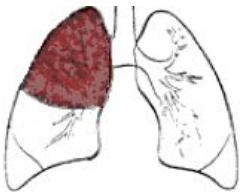
- Group D, aka Enterococcus
- Part of normal flora of GI-tract
- Prey on compromised individuals

Viridens streptococci

- Found in oral cavity of health individuals
- Can cause endocarditis in individuals with damaged heart valves

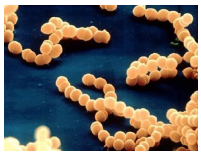
Streptococcus pneumoniae

- Also known as pneumococcus
- Polysaccharide capsule has antiphagocytic properties
    - ~90 distinct capsular serotypes
- Found in naso-pharynx of healthy individuals
- Can cause
    - lobar pneumonia
    - meningitis
- Prevention strategies (elderly, alcoholics, crowded living, vaccination)



Gram Negative Cocci

NEISSERIA MENINGITIDIS



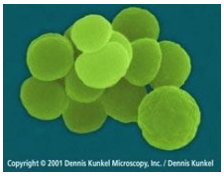
- Gram negative diplococci
- Laboratory isolation using chocolate agar, 5-10% CO2, 37 C
    - use selective media (i.e., Thayer-Martin) when isolating from nasopharynx
- Frequently found in the naso-pharynx of healthy individuals
- Antiphagocytic polysaccharide capsule
    - 13 different serogroups
    - A, B, C, X, Y and W135 most prevalent
- Carriers can occasionally develop infection or pass organism to non- immune individuals who develop infection
- Only infects humans!!!
    - usually children or those living in crowded living quarters
    - occasional epidemics
- Infection can result in
    - Meningitis
    - Septicaemia (starts as skin rash)
    - Waterhouse-Friderichsen Syndrome (complication of septicaemia…most severe form of septicaemia by N. meningitidis)

- …first described in 1894 by Arthur Francis Voelcker (1861-1946)
- …then in 1901 by the British dermatologist Ernest Gordon Graham Little (1867-1950).
- It was first reported as an entity by Waterhouse in 1911, and the subject was comprehensively reviewed in 1918 by the Danish paediatrician Carl Friderichsen
- ….so it was called **Waterhouse-Friderichsen syndrome**…

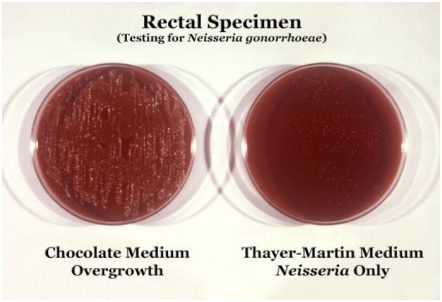
Prevention and Treatment

- Penicillin is primary antibiotic used
- Vaccination is recommended for children (11-12 years), teenagers and college/university students living in dormitories
    - Conjugated vaccine for serogroups A, C, Y and W135
    - Now have meningococcus vaccine for infants at 2-5 months (serogroup C)

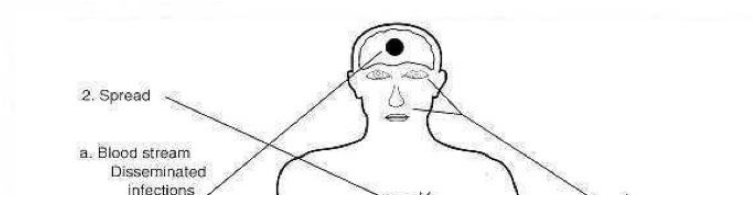
Neisseria gonorrhoeae



- Gram negative diplococci, 0.6-1µm in diameter
- In a clinical lab, grow on Thayer-Martin plates, in damp environment with CO2
    - VERY sensitive to drying and changes in temperature
- Causative agent of STD gonorrhea
- In US, it is the second highest reported STD, after chlamydia
    - >350,000 cases/year reported in the US (2001)
    - Number of cases is now decreasing every year



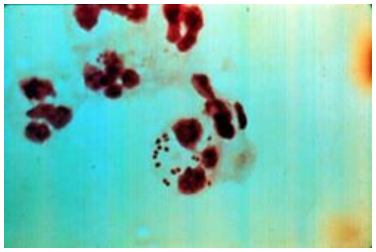
- Clinical gonorrhea
    - **MEN**: causes acute infection of urethra (90-95%)
    - **WOMEN**: 50% are ASYMPTOMATIC!!!
        - Cervicitis
        - If untreated can cause PID, sterility
- Disseminated Gonococcal Infection (DGI)
    - 1-3% cases, usually women
    - Fever, skin infection, arthritis
- Neonatal infections
    - Rare, but newborns can acquire infection from mother during birth
    - Causes gonococcal ophthalmia neonatorum (acute purulent conjunctivitis)



Clinical manifestations of N. gonorrhoeae

N. gonorrhoeae

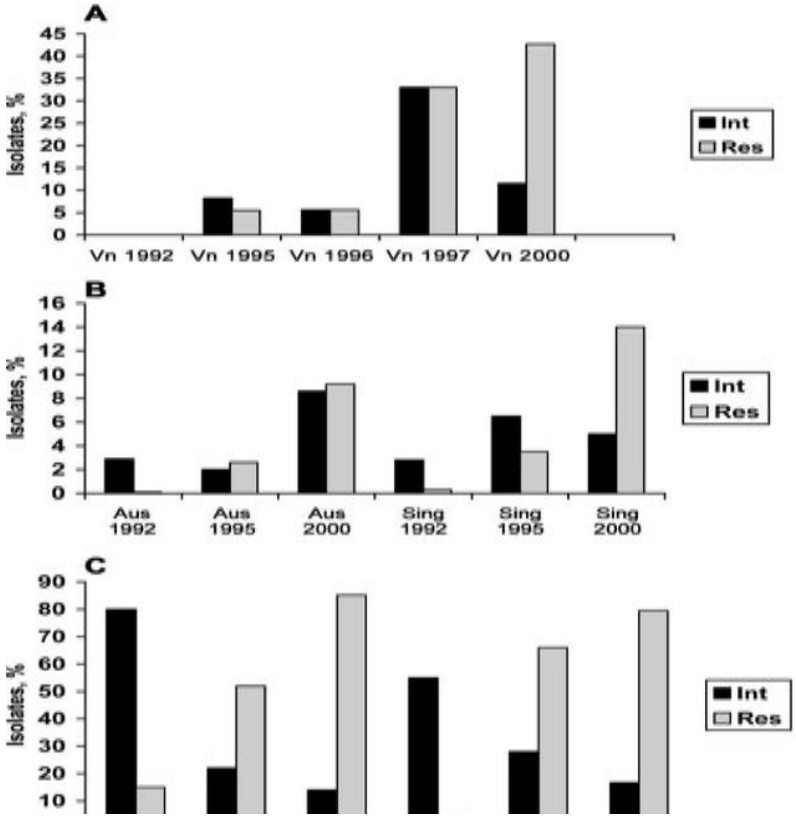
- **DIAGNOSIS**
- **MEN**: use microscopy to directly observe swabs of urethral discharge
- **WOMEN**: culture is necessary from endocervical, urethral and anal swabs



Urethral smear with intracelllar G-ve diplococci

PREVENTION and TREATMENT

- Penicillin resistance is emerging (South-East Asia, West Africa, Canada and US)
- Treat using ceftriaxone, cefixime, ciprofloxacin or ofloxacin combined with doxycycline/azithromycin
- Resistance to ciproflaoxacin (quinolones) emerging
- SIMULTANEOUS treatment of partners is ESSENTIAL
- No vaccine available



Evolution of quinolone resistance in selected countries in the World Health Organization Western Pacific Region, in A, Vietnam (Vn); B, Australia (Aus) and Singapore (Sing); C, China (Ch) and Hong Kong Special Administrative Region (HK). Int, intermediate, less susceptible to ciprofloxacin (MIC, 0.12-0.5 mg/L); Res, resistant to ciprofloxacin (MIC, 1 mg/L) (Antibiotic Resistance in Neisseria gonorrhoeae, J.W.

Tapsall, Clinical Infectious Disease, 2005)