HSS notes midterm 1

Microbiology and Immunology (University of Ottawa)

Lecture 1

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

Microorganisms in the news?

- Increase in bacterial resistance
- Virus and low sunlight raises multiple sclerosis risk
    - How does increase vitamin D and or vaccinate/control EBV?
        - Attacks the myelin sheet; antibodies
        - Recognize Myelin sheet as foreign and then we attack it
        - Antibiotic resistance

Correct way to address these "germs"

- Genus - always make sure to capitalized
    - Listeria Monocytogenes
    - Listeria species
    - Listeria
    - Listeriors

underline any bacteria

Different types 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 !!! - (i.e., not "germs", "bugs")

What are they made out 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…
    - We have bacteria in our GI
    - Ferment; help with metabolism
    - Food spoils faster than the bacteria that can grow and kill you
- Can we live without bacteria?
    - No, there recycle so its not just us using them; the environment around you as well
    -

"Normal" flora...the good guy

- Resident versus Transient
    - Resident; the bacteria that we have once we are born
        - During labour partly
        - Most in the 1st year of life
            - What we eat
            - Environment that we are exposed to
    - Transient: the bacteria that can come and go
- GI-tract: colon is inhabited by anaerobes and coliforms
- Skin: mostly coagulase-negative staphylococci
- Where should there be NO bacteria?
    - Bloodstream
    - Brain
    - Central nervous system
    - Private parts
        - Good bacteria; flush stuff out; protect; take up stuff so bad things can't come; balance pH
    - eyes
- What can they do for us?
    - protection from invasive (bad) bacteria
    - metabolism (vitamin K), immune stimulation

What protects us from bad guys

- Mechanical barriers
    - skin, saliva, mucus, tears, hairs, etc.
        - Skin is the most protective
- 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
    - Attach to something (e.g. ligand receptors present on surface of bacteria)
    - This is a prerequisite for the initiation of infectious process
- Toxin production (destroys some of our defences)
    - Bacteria may produce endotoxins that damage host cell, and host defense against this is antibodies
- Opportunism
    - Normally bacteria won't cause disease; but if they go where they are not supposed to go they can cause problems
- Compromised host (how does this happen?)
    - Immune system not strong
    - Drugs
    - Poor diet
    - Stress
    - Genetics
    - Infectious diseases
    - Age
- bacteraemia versus septicaemia? (aemia = blood)
    - Bacteraemia: bacteria in the blood
        - A type of septicaemia
    - Septicaemia: blood poisoning
        - Variety of types
        - Result of other things such as drugs

Infectious Disease and the Human (Immune) Response

Microbial disease

- Interaction between microorganisms and the host (us) - is continuous battle
    - They need to enter-live-multiply
        - Transmit elsewhere to continue growing
- 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] - pathogens are being deposit there without having to do any work
- Clinical disease = easy to recognize
    - Have symptoms that are obvious
- Subclinical infection = hard to diagnose (no symptoms)
    - More problematic
    - No symptoms but we might be carrying pathogens; easy to transmit

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

- Pathogenicity = ability to produce disease
    - Non pathogen; not able to cause disease
- Virulence = relative capacity to cause damage (i.e., the degree of pathogenicity)
    - How well it can cause the disease
    - More symptoms, kill patience faster
- Opportunistic = do not normally cause disease but can do so when defense mechanism(s) of hose is breached or compromised
    - Bacteria in the blood because of an infected needle; good bacteria or even bad one can cause opportunities
    - Burn or instrumentation

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
    - Present of antibodies and defence mechanisms
    - Whether or not you are a host; parasites

- Transmission: the transfer of an effective challenge amount from the source to the host
    - Routes of entry: inhalation, ingestion, break in protective barrier, direct deposit
        - Inhalation and ingestion are the top 2
        - Come in contact with them
            - Major one is respiratory and inhalation
            - Ingestion
    - Pathogenicity: capability to inflict damage as a result of invasiveness and toxigenicity
        - Able to cause disease or not and then the chances of it (Virulence)
    - invasiveness (adherence, persistence, avoidance of immune system)
        - the capacity to inflict damage as a result of invasiveness (ability to overcome host defenses and multiply)
        - Adhere to persist on body surface
        - Protect themselves against bactericidal substances present in body fluids
        - Avoid ingestion and destruction by phagocytes
    - toxigenicity (ability to make toxins/production of toxins)
        - Toxins are substances produced by bacteria that damage host tissues or upset systems vital to the host. 2 classes: endo and exotoxins
            - Exotoxins: proteins made by living bacterial cells
            - Endotoxins: toxic substances associated structurally with the bacterial cell and liberated only when cell disintegrates

How does a pathogen adhere to us?

- A bacteria needs to adhere, evade and invade the host
- Tools used to achieve these huge objectives:
    - Surface structures (pili, fimbriae)
        - Adhere to specific receptors present on body cell surfaces
    - Capsules (polysaccharides, protect microorganism against leucocytes)
        - 2nd coat that some bacteria are able to generate and wear
    - Enzymes
        - May contribute towards the virulence of the pathogen that elaborates them
            - Coagulase: accelerating blood clotting to form a barrier against leukocytes and body fluids
            - Hyaluronidase: aka "spreading factors" help bacteria by liquefying
        - Allow organisms to survive in order to survive or die

Toxigenicity

- Toxins are substances (usually proteins) secreted by bacteria with the hope to cause damage
- Two classes:
    - Exotoxins:
        - Bacteria has to be alive bind to certain receptors
        - Excreted by living cells
        - Specific affinities
        - Thermolabile
        - Potent
    - Endotoxins
        - liberated when cell wall disintegrates
            - Bacteria dies, the cell wall breaks up
        - less specific, causes fever, malaise, shock
        - Thermostable
            - Sensitive
            - Resistant to heat, less potent → illegal to have endotoxins in products
        - less potent than exotoxins
            - Not allowed by law to have them in your drugs

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(natural or innate):

    - Specific (adaptive, acquired) - mechanisms aimed at particular infecting organisms are divided into 2 major systems
        - Specific circulating antibodies in body fluids (hormonal activity)
        - Cells trained to attack specific invading organisms (cell mediated immunity)

Innate immunity

- Skin
    - What characteristics of skin make it an effective mechanical barrier?
    - Acid pH; sebaceous secretions and sweat has unsaturated fatty acids (bactericidal)
    - Lower temp that is suboptimal for some bacteria
    - Most important of the innate system
- Mucous membranes (mechanical)
    - Stomach pH: <2
    - Cilia in the respiratory tract
    - Lysozymes (antibacterial substance in e.g. tears)
    - pH - e.g. gastric juice pH, acid pH in vagina, urine
    - Cilia of resp tract eliminate particles bigger than 5 microns (large bacteria carrying dust particles
- Iron binding proteins - (e.g. transferrin/lactoferrin) bind the iron needed for bacterial growth
    - Some bacteria require iron for growth
    - and breast milk that makes iron unavailable
- Phagocytosis
    - PMNs (polymorphonuclear), monocytes and macrophages engulf and destroy bacteria
- Complement

Specific immunity

- Mechanisms aimed at particular infecting organisms
- Humoral and cell-mediated (CMI)
    - Presence of circulating antibodies which are modified serum globulins, physico-chemically tailored to react with specific chemical components to previously encountered invading organisms and produced only in response to these encounters
- What is the difference between innate immunity and adaptive immunity?
    - Innate: protects against ANY invader, does not discriminate
    - Adaptive: directed against one type of invader, dependant on past exposure

Where do immune cells come from

B cells: to make antibodies, need to be told to
TH2 will bind to B cells to make antibodies

Humoral immunity

- Circulating antibodies
- Clear part of blood
- Antibody: a protein that binds specifically to a substance (its antigen - the specific thing it binds to)
    - Igs or immunoglobulins
    - Produced by B-lymphocytes upon stimulation from antigen-presenting T-cells
    - Recognize toxins, capsules, some viral proteins
- Antigen
    - "Non-self" - antigens that we have, we want it to recognize a cell that is not ours
    - Protein, glycoprotein, lipoprotein, polysaccharide
    - What structures could be "antigenic" in bacteria? Virus?
        - The pathogenic mechanism involves production of toxins or presence of a capsule and some viral infections

Antibody binding: how does it occur?

Because antibody is shaped like a Y, will bind to another thing that is shaped like a y -- called clumping or agglutination -- useful because it signals our wbc to say there is a foreign substance → need help

Immunoglobulins (igs) a.k.a antibodies

- Antibody: Ig produced in response to stimulation by an antigen and reacting specifically with it
- Distinguish "non-self" from "self"
- Constant and variable region
    - Variable region is responsible for antigen recognition

Classes of igs

- 5 classes: IgG, IgA, IgM, IgE,IgD
- IgG
    - Host defence
    - Crosses placenta and protects newborn
    - 2 combining sites that combine specifically with antigens like lock and key. Rest of mlcl bind to phagocyte and macrophages, and eventually destroy microorganism
- IgD
    - Role is unknown

Classes of igs cont.

- IgA

    - Host defence
    - Found in secretions
        - Tears, saliva, milk, respiratory, GI and genito-urinary tract
        - Breast feed to give IgA to babies
    - Dimer - 2 units joined together
- IgM
    - Host defence
    - Main globulin produced in early immune response – Pentamer - 5 units joined together
    - Doesn't cross placenta
- IgE
    - Hypersensitivity (allergies) – Defends against parasites

1° and 2° immune response

- 1º Response
    - Ab production triggered on first antigen introduction – Latent period of several days
    - Circulating antibody detectable after 5-10days
    - The antibody in serum is maximum at ~21 days, then drops to low levels
    - First introduction of an antigen into body triggers the production of antibodies against the foreign substance - primary response
        - lag (latent) period up to several days
        - Circulating antibodies detectable in 5-10 days
        - Serum antibodies peaks at about 3 weeks then level drops (eventually to undetectable levels)
- 2º Response
    - Basis for Immunizations
    - Occurs when Ag is introduced 2nd, 3rd,4th …time – Lag, rapid Ab increase (2-3 days), slow decrease – Booster injections to maximize Ab levels
    - Immune response happens when antigen is introduced for the second, third time, etc
    - After a short lag, the concentration of the antibody rises rapidly (2-3 days) and then decreases over time over a much longer period, stabilizing at a lower level.
    - This response can be repeated many times until antibody level reaches a max, usually after 3-5 injections of antigen called booster antigens

Antibody detection

- Serological Reaction
    - Detects the presence of antibodies in serum sample
    - Antigen and antibody interact; agglutination
    - Antibody titration
    - Detect unknown microorganisms using known antisera
    - Clear stuff on slides, put antigens, look for clumps → able to identify what's making us sick
    - IgN: just recently sick, never seen pathogen
    - IgG, IgA: pathogen which you have seen before

Cell-mediated immunity (CMI)

- T-cells **NOT** antibodies!
    - Helper, suppressive, cytotoxic (killer) generated from memory T-cells
- Exposure to antigen induces a response from trained T-cells
- Essential for defence against intracellular organisms, parasites, tumours and other foreign cells (i.e., transplants, grafts)
- Immune-suppressive medication for transplant recipients
- Antigen induces production of 'trained' cells active against that antigen or any organism that carries it.
- Soluble antibodies are not involves
- Cell mediated immunity is based on a large number of T-cell subpopulations and a complex system of interactions
- CMI is active in most microbial infections and is essential in defense against intracellular organisms (and viruses) as well as the defence against parasites, tumor cells, and foreign cells (transplants and grafts)

How does immune system know how to process an antigen

… or… in other words …

- How does your body know whether to activate humoral or cell-mediated immunity based on the antigen it sees?
    - Antigen-presenting cells
    - Will present antigen to their a th1 or th2 drive the appropriate response

The general role of 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 the surface of APC and binds a special receptor on T-helper cells
    - ❏ Chemicals (interleukin, etc.) help direct the response
    - ❏ Receptors are either MHCI or MHCII (major histocompatibility complex)
    - ❏ T-helper cells are Th1 (release macrophages) or Th2 (parasite defense help B-cells - promote immunoglobulin E productions) and we know what that means

Pathway of the specific immune response

Disorders of immunity

1. Allergy and Hypersensitivity
    - OVER-reaction to antigens in the absence of true infection
    - Can be fatal…..ANAPHYLAXIS
    - Too much IgE → causes it to clump
    - We don't want it to bypass yr normal immune system so it is not good to 'boost immune system'
2. Auto-immune diseases
    - The 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" - we want to control this
    - Control by immune-suppressive medication
    - What happens when you suppress immune system: susceptible to everything

Immunization

- Passive Immunization
    - administration of a preformed antibody against a *specific* microbial agent
        - Not going to ask TH2 to help
        - Will give the antibody; can be more than one type
    - IgG animal origin: short-lived, risk of hypersensitivity reaction (IgE antibodies)
    - 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

- Stimulates the immune system by administration of antigen (vaccine)
- LONGER LASTING - memory
- Live-attenuated vaccine
    - Sub-clinical or mild illness mimicking the disease – Local (IgA) and humoral (IgG) immunity
    - Beat antigen, make sure the same shape of antigen is present
    - Rapid immunity development
    - Serious illness in immunodeficient individuals
    - E.g. polio: a person who got vaccinated for polio got polio because it wasn't broken down in the lab
- Killed vaccines, subunit vaccines and toxoids
    - Antigens without infectivity

    - If you kill salmonella, make sure its antigen is still present so the body can get accustomed to it
    - May require boosters –
    - Adjuvant with toxoids
    - Polysaccharide vaccines can be conjugated to protein (see conjugate vaccines)
- Recombinant vaccines
    - DNA recombinant technology – avoids the possibility of live virus surviving the inactivation process
    - Attenuated microorganism
    - Hep B vaccine
- Adsorbed vaccines
    - Vaccine mixed with inorganic salt for slower adsorption and longer-lasting immunity
    - Tetanus, Diphtheria
    - Triangle antigen we need for salmonella, mix in organic salt which causes antigen to hang around longer
- Conjugate vaccines
    - Designed for poorly antigenic microorganisms
    - Conjugate antigen of interest to immunogenic, non-toxic protein – *Haemophilus influenzae* type b
    - Contains a capsule that is poorly antigenic → antibodies against this capsule protects against infection → capsular material is attached to an altered non-toxic protein → capsule becomes immunogenic (able to produce immune response)
- Combined vaccines
    - For ease of administration - attenuated/killed vaccines/ subunits vaccines and toxoids combined together
- Combined Active-Passive Immunization
    - Immediate protection after possible exposure to the microbe
    - Hyperimmune Igs and vaccine injected at DIFFERENT sites – Tetanus, Rabies, Hep B
    - Passive immunization + active immunization → more protection, and make sure to give him 2 needles (scenario is person is going to another country) on each shoulder
        - If you give him the antigen and antibodies at one place, they will clump with each other , therefore give it in different locations

Antibiotic resistance

Introduction

- The first antibiotic (?)
    - discovered in 1929 by Sir Alexander Fleming
- World WarII
    - penicillin used to treat staphylococci and streptococci (1946)
- How effective was penicillin?
- Resistance to penicillin recognized almost immediately
    - 80% of all strains of *Staphylococcus aureus*

    - *Streptococcus pyogenes* (Group Astrep) still treated with penicillin
    - Interestingly, penicillin has never been effective against Gm-negatives (*Salmonella*, *Shigella*, *Bordetella pertussis*, *Yersinia pestis*, *Pseudomonas*) – why?
        - **Penicillin** is **effective** only **against Gram-positive bacteria** because **Gram negative bacteria** have a lipopolysaccharide and protein layer that surrounds the peptidoglycan layer of the cell wall, preventing **penicillin** from attacking.
- The late 1940s and early 1950s?

Antibiotic therapy

- Effective chemotherapy depends on selective toxicity
    - good against pathogens, does not affect the host…
- Exploit pathogen processes not seen in humans
    - cell wall, metabolism, etc.
- We have good bacteria but don't want to kill these
- Knowledge of likely microorganisms is crucial…
    - site
    - organism
    - allergy to host?
- Other considerations…
    - route of administration - topical, ingestion, IV
- Monitoring therapy
    - Assume u don't die in 2 weeks and assume u write the right prescription for the person
    - Monitor how effective antibiotics are → need to have follow-up!
- Adverse effects
    - GI-tract, skin, haemopoietic system, renal system, liver

Acquired resistance

- Previously susceptible organisms, becomes resistant/no longer inhibited by antibiotics
- Three major mechanisms of resistance
    - Alteration in drug target:
        - Bacteria can change its shape --> antibiotic will not recognize receptors, will not be affected
        - Bacteria no longer able to bind, protein shape still functional for the bacteria
    - Bacteria produces inactivating enzymes: break up or inactivate the bacteria
        - Release enzymes that resist/inactivate antibiotics (eg. beta-lactamase)
    - Decreased uptake of antibiotic:
        - Can make cell wall less permeable
        - Produce efflux proteins, pump out antibiotic out of the cell

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 mechanism of action (inherent or"natural")
  - Predictable, based on the way antibiotic acts and the innate characteristics of the microorganism (ie. insensitivity)
- acquired - new or added (driven by two genetic processes in bacteria…mutation and selection(vertical evolution), and exchange of genetic material (horizontal evolution)
  - Previously susceptible organism, becomes resistant/no longer inhibited by antibiotic
  - Something can happen for one of the 3 mechanisms to activate

The chromosomes: role in antibiotic resistance

- Mutations lead to
  - Change the site of the antibiotic target (but protein for bacteria still works fine!)
- Regulatory genes
  - turn on an alternative path
  - turn on efflux mechanisms
- Change cell permeability
  - Become less permeable for ex

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?
  - Day care centres: lots of crowding, resp infections (inhalation and injection),
  - Senior care
  - Animal feed

Decreasing antimicrobial resistance?

- Withhold antibiotics
- self-limited viral infections (i.e., the "common cold")
- Use narrowest spectrum antimicrobial agents
- The base decision about the broadness of empiric antibiotic coverage on the severity of illness
  - clinically stable and not at risk for significant morbidity…maybe appropriate to wait for 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

Lecture 3

Diagnostic Microbiology

- Isolation of pure culture from specimen: must isolate clinical specimens from other species to...
    - Why?
        - Determine strain
        - Study resistance and survival
        - Why patient is sick, what treatment to use
- Culture media: nutrient material used to grow and isolate microorganism.
    - Type used is based on
        - Source of sample
        - Species suspected to be in sample
        - Nutritional requirements of the suspected organism
- Inoculation methods - streak, spread, pour
    - Streak
        - Take inoculating loop (circle holds 15-20 um) and streak the specimen without overlapping
        - Then turn plate, starting by overlapping with the last loop
            - Goal: to dilute sample until you get a single observable colony
        - Can be used for antimicrobial testing
    - Spread
        - Useful to grow number of bacteria in sample
        - Make a dilution, spread cells evenly around plate to be able to count colonies
            - Dilution allows colonies to be separated enough to count
    - Pour
        - Add agar, swish around, and put in in incubator
        - Problem: agar is not to be ~40 deg. C. but if it's too hot, might kill bacteria; colonies become embedded inside agar
    - Inoculated media must then be incubated at appropriate temp to allow organisms to grow and multiply

Preservation of Cultures

- Pure cultures of bacteria are stored
    - Freeze - dried (lyophilized)
    - Frozen at -80℃
- Why would we want to keep a “copy” of a bacteria we isolated from a patient? To keep information on the bacterium
- Short term versus long-term
    - liquid nitrogen (-195oC)
    - freezers
    - lyophilization (freeze-drying)

        - Machine sucks out the moisture and leaves a powder which can be stored at room temperature

Identification

- **Morphology helps to classify and identify, as well as give clues to how microorganisms behave in environment**
- Now that you have a pure culture…
    - colony morphology
        - Form
        - Elevation
        - margin
    - cellular morphology
- The microscope is your friend
    - resolving power of microscope(resolution) is ability to distinguish two closely located objects as separate, distinct entities

Identification - staining techniques

- Generally, three steps:
    1. Make a smear
    2. Fix dried smear by heat
        - Pass it over flame 4-5 times
        - Denatures proteins on the cell wall, acts as a glue to keep sample on plate (won't get washed away when it is stained)
    3. Stain with desired dye

Simple vs. Differential Staining

- Simple stain
    - Useful in an emergency clinical setting
    - single dye normally used
    - all organisms same colour
    - size, shape, number, arrangement, etc.
- Differential stain
    - Differentiate between diff types of microorganisms
    - two or more dyes
    - differences between 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).
    - Stains everything, whether gram + or -
2. Flood with Gram's iodine. (Wash with water).
    - Makes a complex with crystal violet and attaches to inside of the cell wall
        - Binds to peptidoglycan

3. Carefully decolorize with 95% ethanol. (Wash with water).
    - This third step is the most critical and also the one most affected by technical variations in timing and reagents.
    - It not timed properly, will mess up whole stain
    - Gram negative will become invisible
    - Most affected by technical variations in timing and reagents
    - If this is done for too long, will strip even the gram positive bacterium and get wrong results
4. Flood with safranin (pink color). (Wash with water). Air dry, or blot with absorbent paper
    - Gram negative will be pink while gram positive remain purple

Cell wall is the key

- Essential for cell growth and division
- Shape of bacteria related to peptidoglycan layer
- Gram negative usually thinner than Gram positive (which will have thick layer of peptidoglycan but no cell wall)

Other Staining techniques

- Endospore
    - Malachite green applied with heat to penetrate spores followed by counter-staining with safranin
- Capsule
    - Treat with copper sulphate before staining to visualize capsule as clear zone surrounding cells
- Flagella
    - Use of mordant to thicken flagella before staining to visualize them

Fluorescence Microscopy

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

Electron Microscopy

- Electron beam (instead of light)
- Million times magnification possible (0.003 μm)
    - TEM (stain with heavy metals)
    - SEM (3-D image of cell surface)

So what's the bottom line?

- Morphology helps to classify and identify
    - Gram stain
- Gives clues to how they behave in environment
    - 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)

With what do we grow bacteria?

- Salmonella typhimurium
    - Gram negative: growth
      Lactose fermentation: negative
    - (colourless colonies)
- Escherichia coli
    - Gram Negative: growth
    - Lactose fermentation: positive
    - (pink colonies)

Definitions

- Chemically defined – exact composition known and can be controlled
- Chemically undefined – natural products added to some media, so some components can't be controlled (beef extract, blood, etc.)
- If solid (versus liquid) growth – 1.5% agar used
- Enrichment media – increase # of specific bacteria in sample by favouring growth of interested species
    - E.g. blood agar
- Tissue culture media – for cultivating viruses, derived of plant or animal cells

General Media Requirements

- Bacteria – requirements vary
    - Take advantage of this to be able to tell bacteria apart
- Yeasts – high sugar and lower pH
- Anaerobes – must remove oxygen

- All viruses

Selective, differential and S/D media

- Selective media – enhance growth of one bacterial species or suppression of another
  - E.g. separation of guys from girls
- Differential media – differentiate bacteria based on their nutritional requirements and phenotypic characteristics
- Selective / Differential media – very useful in clinical labs (e.g., MacConkey agar)
  - MacConkey agar
    - Allowed gram negative to grow and allows you to determine which gram colony is there - e.g. if colony can ferment lactose, will turn pink

MacConkey - S/D media
(bile salts and crystal violet inhibit Gram +ves)

- Salmonella typhimurium
  - Gram negative: growth
  - Lactose fermentation: negative
  - (colourless colonies)
- Escherichia coli
  - Gram Negative: growth
  - Lactose fermentation: positive
  - (pink colonies)

Temperature requirements

Temperature Definitions - cardinal temperature changes depending on nutritional content of growth medium. Optimum temp usually close to max. Temperature since enzymes activity increase with temperature

- Psychrophiles
  - grow best at temperatures 15-20℃
  - Problematic in food spoilage
- Mesophiles
  - grow best at temperatures 25-40℃
  - most bacteria belong here
- Thermophiles
  - grow best at temperatures 40-85℃

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

Growth of Anaerobic Bacteria

- Anaerobic jar
- Coy anaerobic chamber
- Aerobic microorganisms includes microbe which grow in standard atm of 21% O2
- Anaerobic microorganisms grow in hair atm but can grow anaerobically
- Microaerophilic microorganisms can use oxygen for chemical reactions

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)

Lecture 4

Gram positive Cocci

Staphylococcus aureus

- "Staphule" means grape greek
- Toxins are quite the problem
    - Cytotoxins
    - Haemolysins
    - Enterotoxin (A-E, G-I)
- Exfoliative toxins (ETA, ETB)
    - exotoxin
    - Toxic shock syndrome toxin 1 (used to be exotoxin C and enterotoxin F)
- Enzymes: allow organism to survive, move around inside the host
    - Coagulase (coagulation of fibrin)
        - Made of almost all pathogenic staphylococci
        - Used in lab 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 or OR, would precaution
    - Educations of health personnel
    - Handwashing!

Staphylococcus epidermidis

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

Streptococci

- Attached in pairs of forming chains
- “Streptos” - green word for twisted
- Subdivided into “groups” based on
    - Haemolytic properties
    - Carbohydrate C antigen
    - M-protein
- Divides beta-hemolytic
    - Mostly group A (strep throat)

Streptococcus pyogenes

- Group A, beta-hemolytic, S. pyogenes causes:
    - Acute tonsillitis (strep throat) - can lead to rheumatic heart disease
        - In developing countries
        - Eventually immune system will destroy step but some antigens on the outside of srep are similar to antigens on the outside of heart (causes immune response against heart)
    - Skin infections like - Impetigo, cellulitis, etc
    - Complications following infection (esp in younger patients) - Fever and septicaemia
- Caused by toxins
    - Streptolysins (O and S)
        - Neutrophils and macrophages
        - endotoxins
    - Streptococcal pyrogenic exotoxins (spes)
        - Scarlet fever rash
            - Tongue becomes red
- Enzymes
    - Hyaluronidase (helps spread of bacteria)
- Virtually all are penicillin G sensitive (vs. S. aureus)
- Education of health personnel

- Aseptic obstetric procedures (puerperal fever aka doctors plaque)
    - Dr.s would be in the morgue then deliver a baby, thereby giving injection to mother
- Early detection and treatment

Flesh-eating disease...aka necrotizing 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…
    - Strep becomes infected by Bacteriophage, which has has gene encoding for enzyme allowing bacteria to escape entrapment and killing by neutrophils (WBC)

Streptococcus agalactiae

- Group B
- Found in vagina of healthy women (can cause neonatal infections)
    - Early septicaemia
        - Respiratory distress for shock at birth
        - High fatality rate (serious)
    - Delayed meningitic form
        - 1-12 weeks postpartum
        - Sequelae - neurological damage may result

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

- Aka pneumococcus
- Polysaccharide capsule has antiphagocytic properties
    - ~90% distinct capsular serotypes
- Found in naso-pharynx of healthy individuals
- Can cause
    - Lobar pneumonia - auto infection, more frequent in babies, alcoholics and elderly
    - Meningitis - often in babies and old people
- Prevention strategies (elderly, alcoholics, crowded living, vaccination)

Gram negative cocci
Neisseria meningitidis

N. 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 naso-pharynx of healthy individuals
- Antiphagocytic polysaccharide capsule
    - 13 different serogroups
    - A C C X Y and W135 most prevalent
- Carriers can occasionally develop infection of pass organism to non-immune individuals who develop infection
- Only infects humans
    - Usually children or those living in crowded in 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 by 1894 by arthur francis voelcker (1961-1946)
- 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 by 1918 by the danish pediatrician 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 we have meningococcus vaccine for infants at 2.5 months (serogroup C)

Neisseria Gonorrhoeae
N. gonorrhoeae

- Gram negative diplococci, 0.601 um 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
    - >350k cases/year reported in US (2011)

    - Number of cases is now decreasing every year

- Clinical gonorrhea
    - Men: causes acute infection of urethra (90-95%)
    - Women: 50% are asymptomatic
        - Cervicitis
        - If if untreated can cause PID, sterility
- Disseminated gonococcal infection (DGI)
    - 1-3% cases, used women
    - Fever, skin infection, arthritis
- Neonatal infections
    - Rare, but newborns can acquire infection from mother during birth
    - Causes gonococcal ophthalmia neonatorum (acute purulent conjunctivitis)

- Diagnosis
    - Men: use microscopy to directly observe sqbs of urethral discharge
    - Women: culture is necessary from endocervical urethral and anal swabs

- 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 ciprofloxacin (quinolones) emerging
    - SIMULTANEOUS treatment of partners is ESSENTIA
    - No vaccine available