1. Properties of Bacterial Pathogens

Professor David Holden (d.holden@imperial.ac.uk)

1. Main difference between Gram + and Gram – bacteria
2. Examples of intracellular and extracellular bacteria
3. Flagella and type III secretion – 2 related bacterial multi-protein machines
4. 2 examples of manipulation of host actin cytoskeleton: bacterial entry and movement
5. 3 mechanisms of horizontal gene transfer
6. Genome diversity and evolution

Bacteria

- Small and unicellular
- No internal membrane-bound organelles
- Generally haploid
- Some are mobile- use of flagella
- Typical size- 1µm
- Cocci- spherical
- Bacilli- rod shaped
- Spirilli- spiral
- Vast majority are harmless or beneficial (COMMENSAL), but some are pathogenic

- Gram Negative (GN)
  Examples:
  - Escherichia coli- there are many different types; some harmless ones in the gut and some pathogenic ones(EPEC - diarrhea, EHEC - dysentry and kidney failure)
  - Salmonella (typhimurium - food poisoning, typhi - typhoid)
  - Shigella (dysentry)
  - Vibrio cholerae (cholera)
  - Neisseria (meningitidis- meningitis, gonorrhoeae- gonorrhea)

- Gram Positive (GP)
  Examples:
  - Staphylococcus aureus (skin diseases, endocarditis, bacteraemia, joint diseases, pneumonia)
  - Streptococcus pneumoniae (pneumonia, meningitis, otitis media)
  - Streptococcus pyogenes (tonsillitis, necrotizing fasciitis, bacteremia, scarlet fever)

- Mycobacteria (MB)
  Examples:
  - Mycobacterium tuberculosis (TB)
  - Mycobacterium leprae (leprosy)

Identifying Bacteria

- Gram stain: distinguishes between two different kinds of bacterial cell walls
- Bacteria are stained with a violet dye and iodine, rinsed in alcohol and then stained with a red dye
- Stain indicates whether bacteria is GN or GP
MCD Microbiology

- **GP:** PEPTIDOGLYCAN in cell wall retains dye- appears DEEP VIOLET

Structure of GP cell wall →

- **GN:** outer membrane resists the dye. The cells absorb counter stain- appear PINK

Structure of GN cell wall →

**Pathogens**
1. COLONIZE in host, e.g. on mucosal surface
2. PERSIST: find unique niche and avoid host defences e.g. resist complement, phagocytosis
3. REPLICATE- using nutrients from the host
4. DISEMINATE throughout tissues (this is the manifestation of disease)
5. CAUSE DISEASE

- **Extracellular (EC)**
  - Replicates outside cells, e.g. Staphylococcus, Streptococcus, Yersinia, Neisseria

- **Intracellular (IC)**
  - Replicate inside cells
  - Initially taken up by phagocytosis, forming a PHAGOSOME- membrane bound compartment. Then there are three pathways:
    - ESCAPE from the phagosome and replicate in the cytoplasm, e.g. listeria, shigella
    - MODIFY the appearance of the phagosome so host cells cannot recognise the foreign pathogen and prevent fusion with lysosomes, e.g. salmonella mycobacteria
    - SURVIVE in the phagolysosome formed when the phagosome fuses with a lysosome, e.g. coxiella

**Motility and Invasion**
Requires two related multi-protein machines:

- **Flagella**
  - Protein structures protrude through membrane, and the rotation of these cause movement
  - Rings of protein subunits form in the inner membrane on the cytoplasmic face
  - This forms a channel for the assemble of other rod-like proteins
  - Rod-like proteins and CAPPING PROTEINS (which integrate the new subunits) form a hook structure- this is the precursor to the flagella
  - The hook is of fixed length. Capping proteins are then lost and new protein subunits are added to form the base of the flagella
  - Protein subunits form the flagella, again involving capping proteins
  - Rotational plate at base of hook structure forces the flagellum to rotate- TORQUE provides the movement

- **Type III Secretion System**
  - Similar to flagella, but DELIVERS VIRULENCE PROTEINS into host cells
E.g. Salmonella invasion of epithelial cells (GN)
- Effectors interfere with signalling pathways and induce actin polymerisation, membrane ruffling and bacterial internalisation – i.e. the bacteria engineer their own uptake into the cell
- Needle tip structure secretes effector proteins through TRANSLOCASE COMPLEX which forms a channel into the host cell
- This provides the transport mechanism for the virulence proteins, e.g. toxins
- The type of effector proteins determine the result of the injection

E.g. Listeria manipulation of actin (GP)
- Listeria - causes food poisoning and more serious diseases in the immunocompromised, elderly and pregnant women
- Invasion: phagocytosis by zipper mechanism, escapes from phagosome using toxins
- Intracellular movement: polymerises actin on one end of bacterium- forming COMET TAILS which drives the movement of the bacterium around the cell
- Cell-to-cell spread: bacterium can protrude from host cell and be engulfed into neighbouring one

Bacterial Genomes
- Encode 500-4500 proteins: approx 90% are “uninteresting” from pathogenic point of view as they have other functions. Pathogenic genes (VIRULENCE GENES) are mainly selected by immune system
- There is unexpected variation in the genomes of similar pathogens (Core genes + accessory genes = gene repertoire)
- The more strains of pathogens you sequence, the number of new genes remains the same, although you would expect it to decline. This is due to STRAIN SPECIFIC DNA
- Accessory genes: genes for pathogenesis and virulence – huge variation

Replication

Bacteria replicate by binary fission→

Horizontal Transmission

➢ Transformation
- The uptake of exogenous DNA and its integration by homologous recombination
- E.g. neisseria, streptococcus

➢ Transduction
- Phage replicates its DNA in bacterium and cuts bacterial DNA into small pieces
- Some bacterial DNA may be packaged ine phage heads. Bacterium lyses and new phage particles are released (LYTIC INFECTION)
- Phage particle injects bacterial DNA into new bacterial cell
- Injected DNA may be incorporated into bacterial chromosome—BACTERIOPHAGE
- **Conjugation**
  - Transfer of plasmid through CONJUGATIVE MATING BRIDGE – enables DNA transfer
  - The vast majority of accessory genes acquired by horizontal transfer from (mainly) unknown sources
  - If DNA confers selective advantage, they remain in the bacterial chromosomes as PATHOGENICITY ISLANDS—giving the chromosome a mosaic like structure of core “housekeeping” genome with interspersed islands that confer virulence properties
  - Pathogenicity islands are the driving force of evolution, but their origin is frequently unknown
  - An incredible source of genetic variation, rapid generation time + selective pressure, approx 1000,000,000 years = EVOLUTION
2. Bacterial Diseases

Professor Chris Tang (c.tang@imperial.ac.uk)

1. What is understood by virulence and infective dose
2. List the potential sources and possible routes of infection
3. Examples of extra-cellular pathogens; Diarrhoea, diarrhoea, septicaemia
4. Outline some mechanisms related to the examples

Virulence

Virulence: The degree of damage an infective pathogen can cause (Quantitative trait)

Commensal: harmless bacteria e.g. flora

Pathogen: microorganism able to cause disease

Opportunistic pathogen: a pathogen that takes advantage of a host with a defective immune system, i.e. causes disease only in favourable conditions

True pathogen: any microorganism that causes disease

Infective dose: the number of bacteria required to cause disease

Bacterial Pathogens
- Carry 10X more bacteria than human cells
- Only 50/10³ of bacterial species we carry cause disease
- Often carry extra DNA sequences that encode VIRULENCE DETERMINANTS. These are often present on mobile elements defined as PATHOGENICITY ISLANDS

Factors contributing to Virulence
- **Tropism** - find unique niche
- **Replicate** using host nutrients
- **Immune evasion**
- **Toxic**, i.e. induce damage
- **Transmission** between host cells

Infection

Potential sources and routes
- **Respiratory**, e.g. TB
- **Faecal-oral**, e.g. cholera
- **Direct contact**, e.g. glandular fever
- **Vector borne**, e.g. malaria

Extra-cellular Bacteria

E.g. 1) Cholera- vibrio cholerae
- Investigation of outbreak of diarrhoea by John Snow in 1854
- Gram negative bacillus
- Sudden onset of profuse, watery diarrhoea – dehydration
- No blood or mucus in stool – RICE WATER STOOL
- Treatment: ORT- fluid replacement
- Access: water supply
- Colonisation: toxin-co-regulated pilus
- Replication
- Transmission: faecal
  - Damage: cholera toxin
  - The gene encoding the toxin is acquired by transduction and is carried on a bacteriophage

**Mechanism**
- Tropism: using flagellum
- Colonise: toxin-co-regulated pilus
- Toxic: cholera toxin- enzymatic (A1) and binding (B5) subunits.
- Disease resulting from localised epithelial infection
- Toxin lands on cell, B5 subunit binds
- A1 subunit ADP ribosylates G proteins maintaining the active GTP bound form
- This stimulates adenylate cyclase
- ATP → cAMP, which opens ion channel
- There is a loss of chloride ions from the cell and water follows, i.e. there is a net movement of saline solution into the GI tract leading to watery diarrhoea

**Epidemiology**
- London 1854
  - point source outbreak
- Peru 1991
  - 3 epicentres over 4 weeks, 1.3 mil cases
- Haiti 2010
  - Earthquake → single site outbreak
  - Flooding of Artibonite and second hurricane led to outbreak of typical SE Asian strain by UN barracks upstream of original epicentre

**Typing**
- Lipopolysaccharide (LPS) reaction with immune sera
- Infection with phages
- Antibiotic profile
- DNA polymorphisms

E.g. 2) Clostridium difficile
- Inflammatory diarrhoea
- Gram positive bacillus
- Spores on one end (making it difficult to clear)
- Cause extremely “patchy” disease, especially in lower GI tract i.e. colon
- Carried by 15-70% healthy neonates (<1 yr)
- <3% healthy adults
- 10-20% hospitalised, especially with antibiotics
- Exposure to antibiotics allows organism to flourish, i.e. it is OPPORTUNISTIC
- Most EXOGENOUSLY acquired; hospital environment, hands
Cytotoxin

- Toxin AB - single molecule but two subunits; enzymatic and binding
- Toxin A enters apically
- Toxin B enters basolaterally
- Toxin AB is endocytosed and processed into an ENDOsome
- The toxin is then inserted into the membrane of the endosome
- On acidification of the vacuole, enzymatic subunit enters the cytoplasm
- The enzymatic subunit toxins then glycosylate G proteins (Rho GTPases), rendering it inactive and inhibiting effect interaction

E.g. 3) Neisseria meningitidis

- Gram negative coccus
- Leading cause of childhood mortality
- 3 seragroups; A, B and C
- Yearly January peak
- No vaccine for B
- Vaccine of group C lead to reduction in C

Mechanism

- **Colonisation**
  - Affects 10-40% individuals
  - Involves sub-epithelial layer
  - Asymptomatic
- **Septicaemia**
  - 10% case fatality
  - Fever
  - Rash (non-blanching)
  - Cardiac depression
  - Coagulation
- **CSF**
  - Neck stiffness
  - Photophobia
  - Vomiting

- Bacteria expresses type 4 pili which is necessary for adhesion
- Binds to CD46 - complement regulatory protein (specific to human host)
- Fulminant: among highest level of bacteraemia in sepsis, and blebbing (circulating levels of LPS)
- Complement is important in innate immunity - x100-500 increase in disease in complement deficient individuals

Bacterial capsule

- Different for different seragroups
- Vaccine - α2-9 linked sialic acid (Group C) - present on endothelium and RBC - binds factor H by copying human cells using proteins instead of sugars - reduces complement activation
- No vaccine - α2-9 linked sialic acid - produced in brain therefore is not recognised as non-human and vaccine would induce “self” destruction
- Has specific molecules to bind transferring (human molecule but higher affinity) to acquire iron used for replication
3. Antibiotic Resistance and Hospital Acquired Infections

Professor Christoph Tang (c.tang@imperial.ac.uk)

Outline the following
1. The scale/impact of the problem- underlying reasons
2. The organisms involved
3. Reasons for the high rate of hospital acquired infections
4. Mechanism of action of some important classes of antibiotics
5. Mechanisms of resistance
6. Some of the approaches to prevent the emergence of drug-resistant bacteria and nosocomial infections

The scale/impact of the problem

Antimicrobial- interferes with growth & reproduction of a ‘microbe’

Antibacterial- commonly used to describe agents to reduce or eliminate harmful bacteria

Antibiotic is a type of antimicrobial used as medicine for humans, animals- originally referred to naturally occurring compounds

misconceptions

- All major antibiotics are based on the beta-lactam ring, and inhibit PEPTIDOGLYCAN synthesis
- Antibiotics (Penicillin) were discovered by Alexander Fleming in 1928
- In the 1970s, the Surgeon General declared that the golden era of antibiotic discovery would lead to the era of infectious diseases to be over
- It was also believe that resistance against more than one class of antibiotics at the same time would not occur
- Horizontal gene transfer was not thought to be of concern
- Did not think resistant organisms would be at a selective advantage
- An example of how these preconceptions were wrong is the discovery and loss of penicillin in treating Staphylococcus Aureus, where by the 1990s resistance exceeds 80% in community strains and 95% in hospital strains (DIAGRAM ON PP)

The impact of resistance

- Only one new antibiotic has been discovered in the last 30 years. Drug companies are no longer interested in antibiotic development as any discovered would be limited to occasional necessary treatments of multi-drug resistant infections such as MRSA
- Drug companies are not looking to develop drugs for “lifestyle diseases” as these will have a longer-term use so will bring higher profits
- Once an antibiotic is introduced, genetic variation will lead to the emergence of resistance (most likely in hospitals) which then introduces resistance into the community- like with E.coli

Antibiotic Resistance

Factors that contribute to antibiotic resistance include:

- A faster replication rate
- Transduction
- Conjugation
- transformation
- production and release of antibiotics into the environment- use in agriculture e.g. growth promoters for cattle and poultry
- medical intervention
  - lines
  - catheterisation
  - intubation
  - chemotherapy
  - prophylactic antibiotics
  - prosthetic material
- dissemination
  - white lab coats
  - stethoscopes
  - computers
  - hospitals as a source of infection- close proximity of different infections gives rise to a more rapid spread and opportunistic infection (hand washing as solution)

Modes of action of antibiotics

- selective toxicity
  - against microbe not host, especially targets cell wall/membrane due to the structural differences e.g. peptidoglycan
  - CIDAL- bacteria lose integrity of cell membrane
- protein synthesis
  - STATIC- prevent replication
  - Specificity of action; ribosomes on prokaryotes are different from eukaryotes
  - E.g. aminoglycosides, tetracycline
- metabolic targets
  - affect pathways leading to nucleic acid synthesis
  - mammalian cells take up PHOLIC ACID in order to synthesise nucleic acids
  - bacteria make their own pholic acids, so antibiotics target pathways before the pholic acid is made
  - in effect they act as competitive inhibitors- an analogue of para-aminobenzoic acid (compound involved in the pathway)
  - e.g. sulphonamides
- inhibition of nucleic acid synthesis
  - e.g. Rifampicin- prevents RNA synthesis
  - quinolones and fluoroquinolones disrupt coiling

Examples

  - Inhibition of cell wall synthesis:  Penicillins (cephalosporins), Glycopeptides (Vancomycin)
  - Inhibition of protein synthesis: Tetracyclines, Aminoglycosides
  - Inhibition of DNA metabolism: Quinolones
  - Metabolic targets: Sulphonamides, Trimethoprim
  - Inhibition of RNA synthesis: Rifampicin
  - Other: Metronidazol
Mechanisms of resistance

When a cell takes up an antibiotic, it usually leads to cell death. However mechanisms of resistance to antibiotics include:

- **decreased antibiotic influx**
- **increased antibiotic efflux pumps**
  - e.g. tetracycline
- **drug inactivation**
  - by enzymes
  - e.g. penicillin; pencillinase, beta-lactamase, NDM1 (a new enzyme which cleaves the beta-lactam ring)
- **target modification**
  - prevents binding
  - e.g. quinolones, penicillin
  - MRSA- Mec element encodes a different penicillin binding protein, i.e. an altered target site, therefore is resistant to cleavage
- **target amplification**
  - e.g. sulphonamides
  - increased coding for enzyme which cleaves the antibiotic, therefore the sulphonamides cannot compensate

Spread of Resistance

- Primarily horizontal gene transfer; conjugation, transduction, and transformation
- Integrons; stretches of DNA which occur in clusters with an inbuilt promoter, and a site for recombination and integration (therefore can take up new resistances)
- Plasmids
  - circular, extrachromosomal DNA
  - replicate on their own
  - transferable between strains
  - move by through conjugation and transformation
  - can assemble many different resistances

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Combating Antibiotic Resistance

- **Hygiene**
  - Monitor and change lines
  - Single rooms
  - Screening for high risk patients or any carrying antibiotic resistance
  - Discharging patients
- **Avoid prophylactic antibiotic prescription**
  - Use guidelines
  - Know local epidemiology
  - Use narrow spectrum antibiotics instead of broad spectrum
  - DOTS (directly observed therapy)
  - Use drug combinations
  - Develop new drugs
4. Fungal Infections
Dr Elaine Bignell (e.bignell@imperial.ac.uk)

1. Name, and distinguish between, the three major types of human illness caused by fungi
2. Summarise briefly the ecology and epidemiology of infectious fungi
3. Define the terms superficial mycoses and deep mycoses, giving appropriate examples of each type of infection
4. Describe briefly the main classes of antifungal agents

Fungi

- Largest group of organisms
- 70,000 species described
- Approx 200 human pathogens
- Eukaryotic
- Independent group equal in rank to plants and animals
- Complex cellular structure - similar to mammalian so difficult to treat
- Genetic material organised into membrane-bound nucleus
- Digest their food extracellularly - hypha are non-motile organisms suspended in their own food source - secrete hydrolytic enzymes which break down biopolymers into soluble products, which are absorbed
- Infection is a result of the diversity of fungal metabolism
- Produce large numbers of spores which are dispersed. Humans are constantly exposed to the spores - commensal organisms and skin colonisers are transmitted by touch

Fungal illness

There are three types:
1. allergies
2. mycotoxicosis
3. Mycoses

Allergies

- Inhalation of/contact with fungal spores may induce a wide range of allergic diseases:
  - Rhinitis
  - Dermatitis
  - Asthma
  - Allergic broncho-pulmonary aspergillosis (ABPA)
- Allergic responses differ by individual and by species, therefore difficult to diagnose
- Accounts for approx 20% of upper respiratory diseases
- Symptoms:
  - Congestion
  - Sneezing
  - Itching
  - Watery eyes
  - Coughing
  - Headache
- Diagnosis
  - Specific IgE blood test
  - immunoCAP
Mycotoxicosis

- a toxic reaction caused by ingestion or inhalation of a mycotoxin
- secondary metabolites of moulds- exert toxic effect on animals and humans (e.g. penicillin) but are non-essential to the fungi
- can cause death depending on severity of exposure
- symptoms:
  - Breathing problems
  - Dizziness
  - severe vomiting
  - diarrhoea
  - dehydration,
  - Hepatic and renal failure 6 days later
- therapy:
  - gastric lavage
  - charcoal
  - liver transplant
- mushrooms have PSYCHEDELIC properties; symptoms of vomiting and nausea, therapy is supportive-correction of hypoglycaemia and electrolyte imbalance

Mycoses

Classified by the level of tissue affected:

- **Superficial**
  - Cosmetic fungal infections of the skin or hair shaft
  - No living tissue invaded
  - No cellular response from the host

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<td>White piedra</td>
<td>Trichosporon beigelii</td>
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<td>Tinea nigra</td>
<td>Phaeoannelomyces werneckii</td>
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- **Cutaneous**
  - Caused by distinct groups of fungi; dermatophytes or keratinophilic fungi → dermatophytes/dermatomycoses
  - Produce extracellular enzymes (keratinases) which are capable of hydrolyzing keratin (in human tissue)
  - Inflammation is caused by host response to metabolic by-products
  - Not life-threatening; respond to oral or topical treatments
  - E.g. epidermophyton, microsporum, C. Albicans, TRYCHOPYTON (athlete’s foot- most common)
  - Infection nomenclature- prefix=tinea (latin for worm), suffix=latin name for place on body
  - Tinea capitis- fungal infection of the skin of the scalp, eyebrows and eyelashes, with a propensity for attacking hair shafts and follicles. Most common paediatric dermatophyte infection
  - Tinea pedis (athlete’s foot)- infects 70% of the population

- **Subcutaneous**
  - Chronic, localised infections of the skin and subcutaneous tissue
  - Following traumatic implantation of the aetilogic agent
  - Rare
  - E.g. sporotrichosis, chromoblastomycosis, mycetoma
Systemic (Deep)
- Primary pathogens: able to establish infection in a normal host, e.g. coccidioides immitis
- Opportunistic pathogens: require a compromised host in order to establish infection, e.g. candida, aspergillus
- Thermally dimorphic fungi, therefore can change form in the host making them difficult to treat
- Exist in natural surroundings, soil, bird and bat droppings
- Limited geographically (Central and Southern America)
- Infection by inhalation, pulmonary involvement, dissemination

Candida Infections

- >100 identified, 6 of which are pathogenic e.g. candida albicans opportunistic commensal
- In immunocompromised hosts, colonisation and invasion of tissues occurs e.g. GI flora compromised – lesions – disease

Superficial
- Usually due to impaired epithelial barrier functions
- Occur in all age groups, but most common in newborn and elderly
- Responds readily to treatment

Mucosal
- Symptomatic
- Elderly and neonates
- Prevalent in HIV patients; oropharyngeal, esophageal, vulvovaginal

Systemic
- Not seen in normal healthy individuals
- Approx 1/3 most common cause of fungal blood infections in ICU
- Very difficult to treat
- Well characterised risk factors; chemotherapy, gut-related surgery, catheters

Virulence processes

- Molecular mechanisms of virulence processes unknown
- The knowledge of enzymes in very important in understanding the mechanisms

The growth environment affects C. Albicans cell shape:
- At pH4.0 and a low growth temperature—yeast form
- At pH6.0 and nitrogen starvation – pseudohyphae
- At pH7.0 in serum – true hyphae form

Stage 1: Colonization
Epithelial adhesion
Nutrient acquisition

Stage 2: Superficial infection
Epithelial penetration
Degradation of host proteins

Stage 3: Deep-seated infection
Tissue penetration
Vascular invasion
Immune evasion or escape

Stage 4: Disseminated infection
Endothelial adhesion
Infection of other host tissues
Activation of coagulation and blood clotting cascades

Virulence factors:
- Adhesins
- Hydrolytic enzymes
- Hypha formation
- Phenotypic switching
- Molecular mimicry?

Hydrolytic enzymes
Hypha formation
Host mimicry?
Immunomodulators?
Invasive Aspergillosis (IA) Infections

- Less frequent than candida
- Major clinical problem
- Aspergillus fumigates and aspergillus nidulans have aerial projections and spores

IPA (pulmonary)

- In compromised host, e.g. chemotherapy, transplants, leukaemia
- Problems with diagnosis; presentation of fever often leads to pre-emptive diagnosis of bacterial infection
- Innate immunity is very important in protection
- Accounts for 60,000 deaths/year worldwide
- 60-80% mortality rate

Diagnosis

- **Sample acquisition**
  - Skin
  - Sputum
  - Bronchoalveolar lavage
  - Blood
  - Vaginal swab/smear
  - Spinal fluid
  - Tissue biopsy
- **Microscopy**
  - “gold standard”
  - Definitive diagnosis based on cell shape
  - Rapid
  - Cheap
  - Dependent on mycologist
- **Culture**
  - Slow
  - Prone to contamination
  - Requires skilled sample collection
  - Positive ID allows susceptibility testing
- **Identification**
  - Requires highly experienced mycologists

Non-culture methods

No specific non-culture methods, but antibody and antigen-based assays exist. These detect:
- Glucan
- Mannan
- Enolase
- Proteinase

PCR can also be used. This is highly specialised so is not used often.
Antifungal Therapy

Targets

- **Cell membrane**
  - Fungi use principally ergosterol instead of cholesterol
  - Inhibit its synthesis by acting upon fungal cytochrome P450 enzymes
  - Polyene antibiotics
  - Azole antifungals

- **DNA/RNA synthesis**
  - Some compounds may be selectively activated by fungi, arresting DNA synthesis
  - Pyrimidine analogues
  - Flucytosine

- **Cell wall**
  - Unlike mammalian cells, fungi have a cell wall
  - Premier target of antifungal therapy
  - Major components are glucans and chitin
  - Non-specific inhibition of β 1,3 glucan synthase
  - Echinocandins

CASE STUDY ON POWERPOINT
5. Viral Properties
Geoffrey L Smith (geoffrey.l.smith@imperial.ac.uk)

1. What are viruses?
   - size, structure and composition
2. How do they replicate?
   - lytic or latent
3. Give examples
   - polio, influenza, HIV and Herpes Simplex Virus

Properties of Viruses

- Small: 20-450 nm
- Obligate intracellular pathogens
- Composition: nucleic acids and proteins (some carbohydrate and lipid)
- Unique mode of replication
- Diversity; all species are infected- some may cause plagues and some may be asymptomatic

Virus Classification

- Family
- Sub-family
- Genus
- Species
- Strain

Example on powerpoint

Parameters used for classification

- Type of disease  e.g. arbovirus
- Mode of transmission
- Structure
- Immunological relatedness e.g. antibody responses
- Nucleic acid sequence
- Protein structure- more generally conserved for a particular function
- Mode of replication

Structure and Composition

- **Nucleic acid**
  - DNA or RNA
  - Linear or circular
  - Monopartite or segmented
  - Double stranded or single stranded
  - + or – polarity
  - 3-1200 kb
- **Nucleocapsid**
  - Helical or icosahedral (20 face)
- **Lipid envelope**
  - In some cases, envelope derived from the cell
- Fragile
- Destroyed by organic solvents, which often inactivates the virus

Examples
- Tobacco Mosaic (TMV) - helical
- Polio - icosahedral
- Influenza - helical enveloped
- Herpes simplex (HSV) - icosahedral enveloped

Nucleic Acids of viruses are also classified according to:
- Size (kb)
- DNA/RNA
- Single stranded or double stranded
- Number of segments, and whether these are diploid, circular etc

Measurement of Viruses
- By observing disease in host
- Plaque assay (infectivity) using virus titrations
- Electron microscopy
- Polymerase chain reaction
- Immunological evidence of infection

Plaque Assay
- Repeating process of infection of susceptible host cell, replication, release and infection of new cell
- This is a series of dilutions, and sample placement onto culture of susceptible cells
- Areas of killed cells large enough to be seen = PLAQUE
- Titres expressed as plaques forming units per ml
- Not all viruses can be titrated e.g. HBV

Virus Replication

Latent Period - the time after infection before infective particles are produced

Eclipse Phase - the time where expression of proteins and replication occurs, but no infective particles are present

Mean Burst size - the increase from the initial and final titre size. The average number of particles released from each cell depending on their metabolic activity
- Binding to host cell; very specific
- Penetration; e.g. fusion of virus and cell membrane
- Eclipse phase; expression of virus proteins and replication of nucleic acids. Highly regulated
- Assembly; production of new infectious particles
- Release; cell lysis or budding (acquiring lipid envelope from cell plasma membrane)

Binding and Penetration

- **Binding**: specific interaction between virus surface proteins and cell receptors
  - HIV gp120: CD4
  - Epstein-Barr virus gp340: CD21
  - Influenza virus haemagglutinin (HA): sialic acid
Penetration
1. Enveloped viruses: fusion between virus and cell membranes
   - HIV and measles at cell surface
   - Influenza with acidified intracellular vesicles
2. Non-enveloped viruses: disruption of host cell membrane integrity, genome or core crosses into cytosol
   - Polio
   - Bacteriophage T4 in E. Coli

E.g. Influenza Virus Entry
1) binding of HA to sialic acid
2) endocytosis
3) acidification of vesicle with H+ leads to conformation change
4) fusion
5) release of RNA into cell

Eclipse phase
- Virus particles disassembles
- No infectious particles present- most of the capsid remains outside cell
- Expression of virus proteins- highly regulated; temporal and quantitative
- Replication of virus nucleic acid

Baltimore Classification of Viruses
- Viruses have a large range of genomes and different replication mechanisms
- All are dependent on host ribosomes for translation, therefore mRNA forms the core of the classification
- Uses how the virus forms mRNA before they make proteins; this means the virus must provide its own RNA polymerase and package it within the virus to bring into the host
- SsRNA +ve can be translated immediately into proteins

Examples of Viruses
- Polio
  - 20nm
  - Icosahedron
- Very stable to acid pH and proteases
- Genome: +ve ssRNA (mRNA); translation into polyprotein, then proteolytic cleavage into mature protein
- 7.5 kb
- Replicated via –ve ssRNA intermediate (complementary)

**Influenza**
- Genome (8 RNA segments) transcribed into mRNAs and cRNAs by virus RNA-dependent RNA polymerase
- mRNAs translated to virus protein
- cRNAs copied into virus RNA (v-RNA)

**Human Immunodeficiency Virus (HIV)**
- Retrovirus
- Replicates via reverse transcription
- Cylindrical
- Enveloped
- 110nm
- +ve ssRNA
- 10kb
- Diploid
- Genome: gag, pol and env. Also regulatory proteins tat and rev
- RNA genome → dsDNA via reverse transcription
- DNA integrates into host DNA (PROVIRUS)
- Provirus may remain dormant, but enables vertical transmission
- Expressed by host RNA polymerase II, to make HIV mRNAs, which is regulated by splicing
- At 5’ end, RIBOSOMAL FRAMESHIFTING occurs- which causes the ribosome to pause and shift one nucleotide from the gag region into pol region to make giant polyprotein
- This is inefficient, and happens rarely- useful as gag is required more and the inefficiency means more is transcribed

**Herpes Simplex**
- Virion: icosahedral capsid
- Lipid envelope
- 130 n
- Linear dsDNA
- 152kb, approx 80 genes
- Replication in nucleus, either via lytic cycle or becomes latent
- HSV1 = cold sores
- HSV2 = genital herpes
- Infection via skin abrasions
- **Lytic replication** is a cascade of gene expression of different classes of proteins
  - Alpha/I.E. proteins: regulatory (1-4 hrs)
  - Beta/DE proteins: replicative e.g. DNA polymerase (3-8 hrs)
  - Gamma/late proteins: structural (7-24 hrs)
  - Each class requires prior expression of proteins of the previous class

**Virus Assembly**

- Protein and nucleic acids are assembled to form new particles
- May be spontaneous, e.g. tobacco mosaic virus
- May be complex and have multiple stages, e.g. vaccinia virus, smallpox vaccine
• May occur at cell surface or internally

**Budding**

• From the cell surface, which has virus glycoproteins on its surface
• The nucleocapsid forms a bud in the plasma membrane, and is released
• Similar to exocytosis

**Latency**

• Herpes virus, e.g. HSV or varizella zoster virus (VZV)
• After lytic infection viruses enter neurones and genomes are maintained as circular DNA (EPISOMES) in nucleus
• May reactivate years later to cause recurrent infections, e.g. cold sores, shingles (VZV)

Geoffrey L. Smith (geoffrey.l.smith@imperial.ac.uk)

Virus Transmission

- Entry, replication and dissemination
- Host is hostile to viruses
- Host non-specific defences
  - Skin
  - Mucous membranes
  - Acid pH
  - Proteases
- Host innate immunity
  - Complement
  - NK cells
  - Phagocytes
  - Interferons
  - Fever
  - Inflammation
  - Apoptosis
- Specific host immune response
  - Antibody
  - Cytotoxic T cells

Factors affecting transmission

- **Particle stability**
  - Enveloped viruses less stable
  - Icosahedral, non-enveloped viruses more stable e.g. FMDV
- **Duration of shedding**
  - Short duration needs higher virus titres to ensure successful transmission
- **Virus concentration**
- **Availability of new hosts**
  - Population size e.g. measles in an isolated community requires new hosts, therefore spikes of infection may be decades apart (i.e. with new generation comes new host)
  - Animal reservoirs (vectors) e.g. yellow fever virus which is involved in both jungle and urban life cycles

Entry routes

- **Respiratory tract**
  - Influenza
  - Mumps
  - Measles
  - Variola
  - Varicella-zoster virus (VZV)
  - Rhinovirus
- **Skin**
  - HPV
  - HSV1 & 2
  - Rabies
- Yellow fever virus
- **Blood products**
  - HIV
  - HBV
  - HCV
- **Genital tract**
  - HIV
  - HSV2
  - HPV16 & 18
- **Alimentary canal**
  - Polio
  - HAV
  - Rotavirus

**Viral Infection**

**Outcome of infection**

Is influenced by several factors:
- Virus dose
- Route of entry
- Age, sex and physiological state of host
  - HBV; greater chance of establishing chronic infection if infected as neonate or as male
  - EBV asymptomatic as child, glandular fever as young adult
  - Chickenpox more severe as adult

**Consequences of Infection (cell death)**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cell type</th>
<th>Disease manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polio</td>
<td>Motor neurones</td>
<td>Paralysis</td>
</tr>
<tr>
<td>Rota</td>
<td>Gut epithelium</td>
<td>Diarrhoea</td>
</tr>
<tr>
<td>HIV</td>
<td>CD4 helper T cells</td>
<td>Immunodeficiency</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepatocytes</td>
<td>Hepatitis</td>
</tr>
<tr>
<td>Rabies</td>
<td>Perkinji cells (cerebellum-brain)</td>
<td>Hydrophobia</td>
</tr>
</tbody>
</table>

**Persistent or latent**

Outcome of infection can be chronic, or can sometimes lead to an acute or fatal disorder

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cell type</th>
<th>Disease manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>Hepatocytes</td>
<td>Hepatitis</td>
</tr>
<tr>
<td>Measles</td>
<td>Neurones</td>
<td>Sub-acute sclerosing panencephalitis</td>
</tr>
<tr>
<td>HSV1 &amp; 2</td>
<td>Neurones</td>
<td>Cold sores, genital herpes</td>
</tr>
<tr>
<td>VZV</td>
<td>Neurones</td>
<td>Chickenpox, shingles</td>
</tr>
</tbody>
</table>

**Cell transformation and cancer**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cell type</th>
<th>Disease manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>Hepatocytes</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>HPV 6, 11</td>
<td>Epithelial</td>
<td>Common warts</td>
</tr>
<tr>
<td>HPV 16, 18</td>
<td>Epithelial</td>
<td>Cervical/penile carcinoma</td>
</tr>
<tr>
<td>EBV</td>
<td>Bcells</td>
<td>Burkitt’s lymphoma, Nasopharyngeal carcinoma</td>
</tr>
</tbody>
</table>
Local or Systemic

- **Local**
  - Replication locally only
  - Shorter incubation period before symptoms
  - E.g. rhinovirus, rotavirus, influenza, HPV 6 & 11

- **Systemic**
  - Multiple phases of replication
  - Virus spreads throughout body
  - Longer incubation times
  - May be spread via bloodstream or nerves
  - Severe infection
  - Clearance more dependent on cellular immunity
  - E.g. chickenpox, smallpox, measles

**Virus Release**

- The organ in which the virus reaches its highest titre tends to be the organ in which the virus is released
- E.g. warts on the skin indicate the virus was released in the epithelium of the skin
- Blood-- HBV, HCV, HIV
- Skin-- HPV, VZV, measles, variola
- Gut-- Polio, rota, HAV
- Respiratory system-- Rhino, influenza, VZV
- Saliva-- EBV, rabies, mumps
- Semen-- HIV, HBV
- Breast milk-- HCMV
- Placenta-- Rubella, HCMV

**HIV: the epidemic**

**History**

- Emerged 1981:
  - CDC report; cluster of opportunistic infections in young men in LA
  - All had T cell dysfunction
  - All homosexual and/or intravenous drug users
- 1982:
  - Spread of disease
  - Indication of blood borne virus
- 1983:
  - Retrovirus isolated at Institute Pasteur
  - HIV cause of AIDS
- 1986:
  - HIV-2 isolated
  - Origin W. Africa
- 1990:
  - 307,000 AIDS cases
  - 9 mil infected
- 1996:
  - >28 mil infected
MCD Microbiology

Alexandra Burke-Smith

Facts

- Approx 14.00 new infections per day
- No vaccine
- No cure
- Without drugs, outcome is death
- Approx 8,200 deaths each day

HIV genome

- Retrovirus
- Subgroup lentivirus
- 110 nm diameter
- Enveloped protein expressing gp120 anchored gp41
- Bind to CD4 helper T cells
- Cylindrical core- CAG antigens, p24, 018
- Diploid +ve ssRNA genome with associated reverse transcriptase
- Genome integrated as provirus (approx 10kb)
- Genes transcribed by host DNA-dependent RNA polymerase II
- Extra regulatory genes expressed by differential splicing

Infection

- Transmission
  - Sexual
  - IV drug abuse
  - Mother to baby
  - Contaminated blood products
- Entry
  - Virus gp120 binds to cells expression CD4 and a co-receptor CCR5(macrophage tropic) or CXCR4(T cell tropic-later in infection)

Human polymorphism in CCR5

- Mutant allele with 32 bp deletion causes termination of protein after transmembrane domain 4
- Mutant does not function as HIV-1 co-receptor
- Only found in Caucasians (16% hetero, 1% homozygous)
- Homozygotes resistant to infection as transmission largely by CCR5 tropic
- Heterozygotes- 16% of pop, but only 10% of HIV population

Pathogenesis

SEE GRAPH ON PP

- Acute infection
  - for 2-3 months
  - Active virus replication
  - Viraemia
  - Temp reduction in CD4 cell count
- Immune response
o CD8+ HIV-specific CTL produced
  o Virus titre declines
  o CD4+ cell counts recover
  o Patient may become asymptomatic

• Over time (up to 15yrs)
  o Virus replication continues in lymph nodes
  o Largely controlled by CTL
  o Continuous virus variation to escape host immunity

• After
  o HIV virus variants escape control by CTL
  o Virus titres increase
  o CD4+ count declines
  o Patient develops immunodeficiency (AIDS), opportunistic infection → death

Outcomes of Infection

➢ Slow progressors
  - 10-15 yrs as HIV patient before developing AIDS

➢ Long term non-progressors
  - Survive as HIV patients without developing AIDS
  - CTL successfully control HIV variants

➢ Rapid progressors
  - CTL fail to control virus titre
  - Within 1-5 yrs, patient develops AIDS

Vaccine

• Target envelope gp120
• Some anti-gp120 antibodies neutralise virus but virus escapes by antigenic variation
• Vaccine cannot be restricted to single strain
• CTL based vaccines
  o Target conserved epitopes in gag, pol and nef (in HIV genome)
  o CTL clear infected cells but do not prevent infection

Treatment

• AZT
  o Nucleoside analogue
  o Incorporates into DNA and blocks further elongation- temp chain terminator
  o Better substrate for reverse transcriptase than host cell DNA polymerase

• DdC, ddI & 3TC
  o Chain terminating nucleoside analogues with some toxicity

• Ritonavir, saquinavir, indinavir
  o Protease inhibitors- Target GIV aspartate protease
  o Prevent cleavage of HIV polyprotein precursors into mature proteins

Approved Antiretriviral Drugs so far are in four categories:

➢ Nucleoside/nucleotide reverse-transcriptase inhibitors
➢ Non-nucleoside reverse-transcriptase inhibitors
➢ Protease inhibitors
➢ Fusion (gp41 inhibitor)
Drug Resistance

- Mutations - due to errors made by reverse transcriptase and RNA pol II
- Over time, virus becomes resistant to single drug
- Change drug ➔ mutations arise again
- Solution: several drugs simultaneously
  - HAART (highly active anti-retrovirus therapy)
  - Widespread use
  - Harder to develop simultaneous multidrug resistance, but expensive
7. How do viruses cause disease? II

Geoffrey L. Smith (geoffrey.l.smith@imperial.ac.uk)

Influenza

How does influenza cause new epidemics?

- Undergoes antigenic variation
- Antigenic drift
- Antigenic shift
- Viruses from other species infect man and acquire human-human transmission

Virus

- Diameter approx 100nm
- Lipid membrane with embedded glycoproteins:
  - HA- binds to sialic acid
  - NA- removes sialic acid; important when virus os leaving host cell
- Core
  - 8 segment complex (nucleoprotein)
  - RNA genome
  - Polymerase
  - Matrix

Haemagglutinin (HA)

- Trimer
- Each monomer, HA1 (globular head) and HA2 (stalk), are cleaved from precursor HA0
- HA1 binds to sialic acid on cells- antibodies to HA block this attachment and so prevent infection
- HA2 contains fusogenic peptide buried in trimer
  - Acidification causes conformational change, exposure of fusion peptide and its insertion into host membrane
- There are approx 16 types- birds are natural hosts for many

Antigenic drift

- Amino acid mutations in the HA → H3
- These accumulate with time
- Continual change; new variants that escape existing antibodies are selected in presence of antibodies
- Less dramatic change than antigenic shift

Antigenic shift

- Co-infection of a human virus and different strain e.g. avian HA with no pre-existing immunity
- Reassortment of segments in nucleus creates new strain
- Danger:
  - If 7/8 derived from human strain
  - 1/8 derived from avian- if this codes for HA
  - Pandemic as new strain

EXAMPLES ON PP
Influenza Pandemics in the 20th Century

**H5N1 Threat**

- Endemic in avian species
- Spread globally by migratory birds
- Transmitted to other birds e.g. in the poultry industry with high density and hence high virus titres
- Has transmitted to man
- Very high mortality in man as no previous immunity
- Human-human transmission not yet evident, but virus may adapt
- ADAPTATION – may be multifactorial
  - Better binding to human cells
  - Better replication in human cells
  - Better escape from human innate immunity
  - Better transmission between humans
- BINDING OF HA
  - The HA binds to sialic acid on the host cell
  - Avian viruses binds better to the alpha2'-3' linkage to galactose than alpha2'-6'
  - Conversely human viruses bind better to the alpha2'-6'
- REPLICATION IN HUMAN CELLS
  - Avian flu polymerase has glutamic acid at amino acid 672 of PB2
  - Replicates well in avian cells but poorly in humans
  - Human flu polymerases has lysine at position 627 and replicates well in both
  - Avian strains that have adapted to man have lysine at 627
- RESISTANCE TO HOST INNATE IMMUNITY
  - Non-structural protein 1 (NS1) confers resistance to interferon-mediated inhibition of influenza virus replication
  - Avian influenza virus strains may be resistant to avian interferon but less resistant to human interferon
  - Adaptations increasing the potency of inhibitory of human interferon will increase the virus virulence

**H1N1 (Swine origin influenza virus)**

- Originated in Mexico, March 2009
- Declared pandemic by WHO in June
- Efficient human-human transmission
- Low mortality rate
- Tamiflu given widely in UK
- Vaccination underway
- GENETICS OF SOIV H1N1
  - RNA fragments come from avian, swine and human origin
  - All viruses were established in swine for several years prior to reassortment
  - HA of current SOIV only has 79% amino acid identity to current human H1N1
  - The HA is predicted to bind to sialic acid with alpha2'-6' so ability to bind to human upper respiratory tract
  - PB2 has avian type amino acids at 627, but potential for adaptation
  - The NA sequence shows that the virus should be sensitive to tamiflu, but resistances have arised
Anti-influenza virus drugs

- **Amantidine & rimantidine**
  - Inhibit virus by raising pH of intracellular vesicles, therefore HA cannot undergo conformational change to enable fusion
  - Drug resistant mutants arise quickly

- **Relenza, tamiflu**
  - Inhibit virus neuraminidase (NA)
  - NA removes sialic acid and thereby prevents the virus remaining stuck to the cell surface and virions from aggregating
  - Prevents dissemination
  - DOH purchased stocks of tamiflu

**Immune Evasion**

- **Antigenic variation**
  - Escape host immune response to infection, either by antibodies or CTL e.g. influenza, HIV, HCV

- **Hiding**
  - Establish a latent infection in which virus proteins are not expressed, so infected cell may not be recognised by immune system, e.g. HSV

- **Express proteins to inhibit the immune response**
  - Block antigen presentation via class I MHC
  - Block recognition by NK cells
  - Secrete proteins to capture cytokines, chemokines or interferons
  - Block intracellular signalling pathways or apoptosis

**Interferons**

- Species-specific soluble glycoproteins
- Induce an anti-viral state in cells bearing receptors
- Type I IFNs (alpha and beta) bind to a common type I IFN-R receptor
- Type II IFN (gamma) binds to the type II receptor
- Type III IFN (lambda) binds to another receptor
- Have direct anti-viral activity and immunomodulatory roles, e.g. IFN- gamma promotes TH1 response
- Many mammalian viruses interfere with interferon
- Interferon and virus countermeasures provide a vivid illustration of viruses (HOST EVOLUTION)

TO SEE HOW INTERFERON BLOCKS VIRUSES, LOOK AT PP DIAGRAMS
Smallpox

- Has been eradicated
- Mortality rate 30-40%
- Centrifugal distribution of lesions distinguishable from chickenpox
- VARIOLATION (1723) – Lady Mary Wortley-Montague
  - Observed practice in Turkey, “engrafting” of sample from lesions into veins
- VACCINATION (1796) – Edward Jenner
  - Using cowpox from milkmaids lead to subsequent protection from smallpox
  - Experiment; exposed child to Cowpox and then small pox, which he didn’t become infected by
- During the 19th century, vaccination spread through the world, with the first freeze dried vaccine in 1950
- Only in 1980 was eradication (certified by WHO) complete; following the intensive eradication campaign of 1967- relied on surveillance on containment with financial rewards as incentives
- Estimated cost of eradication approx $250 mil - is the first and only disease to be eradicated

Why was eradication possible?

- No animal reservoir
- No latent or persistent infection
- Easily recognised disease
- Vaccine effective against all strain
- WHO determination
- Vaccine properties
  - Potency
  - Low cost
  - Abundance
  - Heat stability
  - Easy administration

Why was vaccination effective?

- Outer envelope proteins of vaccine virus (the vaccine) and variola virus (the cause of smallpox) are highly conserved
- Capsid proteins highly conserved (even though only discovered after eradication)
- Understanding of all the antigens recognised by the immune system unnecessary if it works

Viral Vaccines

Historical milestones

- 1885: Pasteur’s rabies vaccine
- 1937: Theiler’s live attenuated yellow fever vaccine (Still used)
- 1943: influenza vaccine
- 1954: killed polio vaccine (SALK)
- 1956: live attenuated polio vaccine (SABIN)
- 1960: measles
- 1966: rubella
- 1967: mumps
- 1986: genetically engineered recombinant vaccine for HBV

Considerations for development

- Which antigens?
- What type of immunity is needed?
  - Antibody or cell mediated
  - Secretory (IgA) or systemic (IgG) antibody
- When is the immunity needed?
  - When does the pathogen induce disease?
  - Exposure in endemic areas
- How should the vaccine be administered?

Types of vaccine

- **PASSIVE: immune globulin, maternal antibodies**
  - Advantage: immediate protection
  - Disadvantages: short lived, serum sickness, immune responses to foreign proteins
  - E.g. rabies and infants born to HBV +ve mothers

- **LIVE: attenuated form of virulent organism, immunologically related organism, virulent organism**
  - Advantages: induce both T cell and antibody responses, long lasting, low cost, ease of manufacture and administration
  - Disadvantages: safety (may revert to virulence, or cause serious infection in immunosuppressed, heat lability, and virus may be shed into the environment

- **DEAD: antigen preparation chemically treated to inactivate infectivity and toxicity**
  - Advantage: safety (as long as the inactivation is complete- disasters with polio and FMDV)
  - Disadvantages: require multiple administration to achieve solid immunity, may require adjuvants, protection for shorter duration, high cost, reduced cell-mediated immunity
  - E.g. whole virus (rabies), specific proteins from virus (surface antigen for HBV, HA for influenza), peptides to important epitopes

Genetically engineered vaccines

- **subunit vaccines**
  - Identify the gene encoding the desired antigen, express the gene and purify the protein
  - Safe, specific, cheap, abundant supply
  - E.g. HBV vaccine- used since 1986- replaced previous vaccine made from human plasma (expensive, limited and potentially dangerous from other infectious agents)

- **Live recombinant organism**
  - Identify the gene encoding the desired antigen, express from a live replicating virus
  - Use the live recombinant virus as the vaccine
  - Simultaneous expression of antigen and delivery to the immune system
  - Enables development of polyvalent vaccine
Treatment

Antiviral chemotherapy

- Must inhibit the virus without toxicity for host
- Fewer targets than for bacteria
- Targets
  - Virus binding to host cell
  - Virus replication (NB enzymes)
  - Virus assembly or dissemination

Anti-herpes virus drugs

- **ACYCLOVIR (ACV) (Zovirax) 1981**
  - Active against HSV1 & 2
  - Apply topically
  - Activity requires virus thymidine kinase and DNA polymerase
  - Nucleoside analogue- once incorporated into DNA it blocks further elongation (chain terminator)
  - Specificity- ACV better substrate for the HSV TK than host cell TK. ACV triphosphate is a better substrate for the virus DNA polymerase than host DNA polymerase- therefore ACV is active in HSV-infected but not uninfected cells

- **GANCICLOVIR & CIDOFOVIR**
  - Nucleoside analogues
  - Specificity due to use by viral DNA polymerase
  - Used for human cytomegalovirus (HCMV) infections
  - Cidofovir must be given intravenously and has some renal toxicity

Importance of viruses

Valuable research tools:
- For study of cell biology and immunology
- For gene therapy
- As new vaccines
9. Vaccination against Bacterial Infections

Ian Feavers (i.feavers@imperial.ac.uk)

1. List the major non-immune host mechanisms
2. List the components of the innate immune system and major antimicrobial mechanisms
3. Explain how an adaptive immune response is involved in defence against bacterial pathogens
4. Describe how infectious agents avoid host defences
5. Explain the difference between active and passive immunisation
6. Give examples of the different types of vaccine presently available and how they are used

Host Defence against infectious agents

Non-immune mechanisms

- Skin; provides a natural barrier to bacterial infection
- Low PH and antibacterial secretions make it a difficult surface to colonise
- We can strengthen this significantly by routine application of soap and water
- Mucosal surfaces are protected by mucus and ciliary clearance

Host immunity

- **Innate immunity**
  - First line of defence
  - Does not require exposure to infectious agent
  - Almost immediate
  - Mediated by monocytes and PMNs
  - Initially an inflammatory reaction

- **Acquired immunity**
  - Requires exposure to infectious agents
  - Takes time to develop
  - Mediated mainly by lymphocytes
  - Other cell types involved, e.g. monocytes and dendritic cells

Vaccines are primarily aimed at eliciting acquired immunity which requires exposure to the infectious agent or its antigens.

Acquired Immunity

- **Humoral immunity**
  - Directly mediated by antibodies (produced by B cells)
  - Plasma cells are primary source of secreted Ig

- **Cell mediated immunity**
  - Not primarily mediated by antibody
  - Mediated by T cells and NK cells
  - Indirectly other cell types may play a role e.g. macrophages

It is important that a vaccine stimulates an appropriate immune response
- E.g. humoral immunity—important for preventing septicaemia
- Cell mediated immunity—important for the prevention of intracellular infections
• Not mutually exclusive with both elements of the immune response playing a role in protection

*Active and passive immunity*

- Active: immunity elicited in the host in response to an antigen
- Passive: immunity is the acquisition of protection from another immune individual through transfer of antibody or activated T-cells
- The purpose of a vaccine is to induce active immunity

**Vaccines**

*Properties of a good vaccine*

- **Stimulates an effective immune response appropriate to the disease in question**
  - Elicit the correct balance of humoral and cell mediated responses
  - Directed to the relevant site within the host
  - Functional, i.e. does what is needed
  - Should elicit this in all hosts, giving life-long protection without repeated doses, stimulate a boostable response and offer protection against all strains

- **Is safe and does not cause adverse reactions**
  - Parenterally administered vaccine must be sterile (both IV and oral)
  - Vaccine manufacturers follow tightly regulated procedures for consistency
  - Avoid use of human or animal origin material
  - Acceptable safety may depend on the recipient, e.g. adult with cancer may try higher risk than routine paediatric vaccination

- **Is inexpensive to manufacture and distribute**
- **Stable**
- **Easy to administer**
- **Simple for both manufacturer and regulatory authorities to control**

**PROVIDES SUBSTANTIAL BENEFIT TO HEALTH AT LOW COST AND LOW RISK**

**Clinical Trials**

- **Phase 1**
  - Primarily for safety
  - Often used to assess immunogenicity
  - Usually small number of adults
  - E.g. Data collected—local or systemic reactions, fever, diarrhoea, vomiting, headache etc

- **Phase 2**
  - Primarily assessing immune response
  - Also used to expand safety database
  - Typically includes all groups likely to use the vaccine
  - Opportunity to investigate the effect of different dose regimes and formulations, and examine laboratory assays for correlates/surrogates of protection

- **Phase 3**
  - Protection studies, usually placebo controlled double blind trials
    - Provide statistically conclusive data for licensure
  - Require good disease surveillance
    - Case ascertainment
Definition of endpoint

Effectiveness studies, sometimes termed phase 4 trials, measure the ability of the vaccine to achieve specific ends. They are scientifically less rigorous, study designs vary and they are not required for licensure. Can help to convince prospective users of the benefits of a vaccine.

There are EU Guidelines of clinical trials

**Vaccine efficacy**

- Determined in Phase III trials (blinded, placebo controlled)

\[ \text{Vaccine efficacy} = 1 - \left( \frac{\text{attack rate in vaccinated group}}{\text{attack rate in unvaccinated group}} \right) \]

- Usually expressed as %

**Herd Immunity**

- Describes the situation when the vaccination of a portion of the population provides protection to unvaccinated individuals
- Works by disrupting the transmission of a pathogen in the population
  - Toxoids will not offer herd immunity
  - Is not relevant when an individual needs protection independently of the population e.g. travellers
- Particularly important for those who are unable to be vaccinated (e.g. the immunocomprimised)
- EXAMPLE: A model disease with reproduction number 3
  - Vaccine introduced with effectiveness of 67% against transmission
  - Even if next generation not vaccinated, marked reduction in the number of carriers
- The ENDEMIC STATE reflects a balance between the transmissibility of the infectious agents and the level of immunity in the population
  - There is an inverse relationship between the basic reproduction number and the proportion of the population susceptible to the disease
  - The entire population can be divided into those who are immune and those who are susceptible to the disease
- HERD IMMUNITY can be modelled mathematically, determining the minimum proportion of the population that must be immunised at birth (or close to) in order for the infection to die out in the population

MATHEMATICAL EQUATIONS TO BE USED ARE ON THE PP

**Vaccine Formulations**

Consist of three elements:

- **ANTIGEN**
  - To stimulate the immune response to the target disease

- **ADJUVANT**
  - To enhance and modulate the immune response

- **EXCIPIENTS**
  - Buffer, salts, saccharides and proteins to maintain the PH, osmolarity and stability of the vaccine
  - Preservative
    - such as phenoxyethanol or thiomersal (contains mercury) may be added to multidose formulations to prevent growth of microbes ones the vial has been punctured
    - Single dose vials or pre-filled syringes do not usually contain preservative
Antigens

Can be divided into five categories:

- **Live attenuated organisms**
  - Contain mutations that affect the ability of the organism to thrive/cause disease in the host
  - Recent method; attenuated isolates have been rationally mutated using targeted molecular methods

- **Killed whole organisms**
  - Simplest to produce
  - Organism is grown then killed either chemically or by heating

- **Purified component vaccines**
  - Dependent upon technological advances; physical and chemical methods of separation
  - Development of recombinant DNA techniques, genomes sequencing and bioinformatics

- **Conjugates**

- **DNA vaccines**
  - Antigen gene is cloned in a vector so that it is expressed from a promoter sequence that is functional in the host
  - DNA is injected, the host expresses the desired antigen and then mounts an immune response

- **Conjugates**

- **DNA vaccines**
  - Antigen gene is cloned in a vector so that it is expressed from a promoter sequence that is functional in the host
  - DNA is injected, the host expresses the desired antigen and then mounts an immune response

- **There has been a marked increase in the number of vaccines for the prevention of infectious diseases.**
- **This has been characterised by an increasing number of purified protein and saccharide component vaccines.**
- **Recently, there has been an increase in the number of licence submissions of vaccine candidates developed using molecular genetic methods, reflecting the technological changes that have taken place in microbiology during the last three decades.**

**Types of Vaccines**

**Live Attenuated Vaccines**

- **Advantages**
  - Antigenic challenge is prolonged
  - Full complement of microbial antigens
  - Immune system challenged at the right site

- **Disadvantages**
  - Complex
  - Hard to define and ensure safety
  - Difficult to license and control

**Examples**

- **BCG**
  - Mycobacterium bovis
  - Phenotypic and genotypic changes occur
  - Effectiveness cannot be definitive as vaccination programmes instituted at the same time as social, economic and public health improvements - although generally accepted as preventative
  - There is variation in the field trials of BCG, as a result of different trial methodology, vaccine variation and regional TB strain variation

- **TYPHOID VACCINE (Vivotif)**
  - Strain Ty21a
  - Caused by the bacterium Salmonella enteric server typhi
Typhoid fever is characterised by a sustained fever, profuse sweating, gastroenteritis, and non-bloody diarrhoea. It is a major global health problem causing an estimated 16-32 million cases annually and up to half a million deaths in endemic regions.

Isolated by NTG mutagenesis
Formulated in enteric-coated capsules
Safe
It is taken orally which ensures a good immune response in the gut. The organisms are lyophilised in enteric coated capsules allowing them to pass safely through the low pH environment in the stomach. The capsules are acid resistant but dissolve readily at neutral pH.

**Killed Whole Cell Vaccines**

- **Advantages**
  - Simple to manufacture (harvest culture and kill) on a large scale

- **Disadvantages**
  - Variable efficacy
  - Complex and ill defined- often reactogenic because of high endotoxin content
  - Contamination with culture constituents
  - Immune response complicated to define
  - Difficult to license and control pertussis, cholera, plague

**Examples**

- Cholera
  - Killed whole cell parenteral vaccine (1920s) -- poor efficacy, short-lived protective response, strain specific
  - Killed whole cell oral vaccine -- good efficacy, safe, oral delivery
  - Dukoral – killed whole cell and CtxB oral vaccine, drink formulation, may also protect against ETEC (enterotoxin E. Coli)

**Subunit or Component Vaccines**

- **Toxoids**
  - Toxin neutralizing antibodies can be sufficient for protection
  - Detoxified toxins e.g. tetanus and diphtheria
  - Most successful
  - Simple to produce
  - Relatively pure
  - Safe
  - High protective efficacy; very immunogenic, appropriate immune response
  - TETANUS TOXIN: neurotoxin causing muscle spasm. The toxoid used in vaccines is produced from filtered culture supernatant, which is treated with 40% formaldehyde at 37 degrees C
  - DIPHTHERIA TOXIN: exotoxin; a polypeptide secreted as a proenzyme which is cleaved into two fragments. Fragment B is responsible for attachment to and penetration of the host cell; Fragment A is the toxic moiety inhibiting protein synthesis and hence causing cell death. toxoid is produced by formaldehyde treatment of culture supernatant.

- **Cell surface components**
  - Proteins
    - Diverse
- Good immunogens
  - Usually safe
  - May be antigenically variable
  - May be phase variable

- Carbohydrates
  - Capsules and endotoxin
  - T cell independent immune response

E.g. Pertussis Vaccine

- Whole cell is effective but has been associated with a number of adverse reactions
- Development of acellular (component) vaccine driven by poor acceptance of the whole cell vaccine
- Components associated with virulence involve:
  - attachment to ciliated epithelium
  - Adherence and complement resistance
  - Toxins
- Acellular vaccines are multicomponent, and safe and efficacious

- Polysaccharide Vaccines
  - Some bacteria produce polysaccharide capsules
  - Generally elicit T cell-independent immunity
  - poor in infants and poor memory and boosting
  - e.g. meningococcal vaccines, pneumococcal vaccines, Vi antigen of S. Typhi

- T cell independent antigens
  - Antigen is large, linear, not readily degraded, and highly repetitive determinant
  - The resulting immune response is not ideal for vaccination. It is typically characterised by poor immunological memory, low avidity antibodies (no affinity maturation) that are less likely to offer functional protection against disease, and in many cases repeated doses rather than boosting can lead to immunological hyporesponsiveness.

Conjugate Vaccines

- Carbohydrate chemically linked to immunogenic protein
- Sophisticated technology
- Expensive
- Highly purified—safe
- Very effective when humoral immunity is required; long-lived, boostable, offer herd immunity
- PREPARATION: various approaches
  - Random activation of high molecular weight of partly size reduced polysaccharide
  - Degradation of the polysaccharide to form active functional groups at both terminals
  - Degradation of the polysaccharide to form active functional group and one terminal
  - Synthesis of a short saccharide chain from readily available chemical precursors which is conjugated directly to the protein

- T CELL DEPENDENT ANTIGENS
  - Naive T cells primed by interaction with APC
  - After priming, T cells interact with B cells
  - Activated B cells differentiate into plasma cells and memory B cells

- Licensed conjugate vaccines
  - Hib vaccine
- Pneumococcal conjugates
- MenC conjugates

- **In development**
  - Group B streptococcal
  - Staphylococcus aureus vaccine

**LOOK AT GRAPHS ABOUT STATS OF THESE VACCINES ON PP**

**Adjuvants**

- Enhance/strengthen the immune response
- Determine whether humoral or CMI arms of the immune system are stimulated
- Direct the Th cell response
- Favour antibody subclasses
- Delivery systems- create a depot of antigen that can be released over a period to maximise the immune response
  - Mineral salts
  - Surface active agents
  - Synthetic microparticles
  - Oil-water emulsions
  - Liposomes
- Immune potentiators- specifically stimulate the immune system to obtain the desired response
  - Toxins and lipids
  - Nucleic acids
  - Peptidoglycan
  - Carbohydrates
  - Peptides
  - Cytokines and hormones
- Often contain PAMPs (pathogen associated molecular pattern), which are recognised by pattern recognition receptors (such as Toll-like receptors) on cells of the innate immune system to stimulate the immune response
- Adjuvants function primarily before an acquired immune response has been induced to influence the nature and potency of that response.
- **Examples:**
  - Mineral salts- Ca salts
  - Emulsions and surfactant-based formulations- MF59
  - Particulate delivery systems- virosomes
  - Microbial derivatives- MPL

**UK Immunisation Schedule**

<table>
<thead>
<tr>
<th>When to immunise?</th>
<th>What is given?</th>
<th>How it is given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>2, 3 &amp; 4 months</td>
<td>Diphtheria</td>
<td>One injection</td>
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<tr>
<td></td>
<td>Tetanus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pertussis (whooping cough)</td>
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<tr>
<td></td>
<td>Polio</td>
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<tr>
<td></td>
<td>Hib</td>
<td></td>
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<tr>
<td></td>
<td>MenC</td>
<td>One injection</td>
</tr>
<tr>
<td>Around 13 months</td>
<td>MMR</td>
<td>One injection</td>
</tr>
<tr>
<td>3 yrs and 4 months – 5 yrs</td>
<td>Diphtheria</td>
<td>One injection</td>
</tr>
<tr>
<td></td>
<td>Tetanus</td>
<td></td>
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<tr>
<td>Vaccinating the immunocompromised:</td>
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<td>-------------------------------------</td>
<td></td>
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<tr>
<td>• Risk benefit analysis</td>
<td></td>
<td></td>
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<tr>
<td>• Protect the vulnerable patient</td>
<td></td>
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<tr>
<td>• Public health perspective - consider herd immunity</td>
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<tr>
<td>• Protect the patient by immunising close contacts</td>
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<tr>
<td>• Consider especially LIVE vaccines</td>
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<tr>
<td>• Decision depends on the nature of the immunodeficiency and the vaccine</td>
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</tr>
</tbody>
</table>

**New vaccines in Clinical development**

- **Meningococcal**
  - meningococcus is one of a small number of Gram –ve species that naturally bleb off outer vesicles of outer membrane. Just like the outer membrane they consist of lipopolysaccharide (LPS) and proteins.
  - Vaccine is made by extracting the OMVs with detergent to reduce the LPS content and thereby make them less reactogenic.
  - Vaccines are more complex than other purified component vaccines
  - OMV vaccines have been evaluated in a number of efficacy studies. In general, protection is variable and strain specific, especially in the very young who are most at risk of infection
  - The predominant protective antibody response is directed at the PorA protein antigen, which is antigenically variable
  - One solution to this problem is to develop an OMV vaccine that contains multiple PorA proteins

- **Pneumococcal**
- **Group B streptococcal**
- **Staphylococcus**

“Reverse Vaccinology” approach: first to identify potential candidate antigens in the computer. The candidates are then over expressed in a suitable expression system.

LOOK AT MORE INFO ABOUT MENB ON PP FOR MORE DETAIL