MIC3032 Notes

Genetic Approaches to Studying Pathogenesis

- Pathogen: an organism capable of causing disease
- Commensal: (normal flora) an organism able to live in association with another without damage
- But in reality, microbes exist along a continuum from primary pathogen to commensal, with opportunists in between

What makes a pathogen?

- Pathogens, like all organisms, must be able to survive and replicate in a particular niche
- But pathogens in particular can often **gain access to, replicate in, and persist at normally privileged (sterile) sites** within the host (e.g. blood, internal organs, CSF, host cells), and cause damage
- Colonisation and interaction leads to host damage and/or dysregulation = disease

HOST-PATHOGEN-MICROBIOTA INTERACTIONS

- Disease occurs when there is a level of damage which results in perturbation of homeostasis (i.e. the ability of the host to keep various conditions within normal levels)
- This damage (disease) is determined by the interaction of the pathogen with the host
- Variability of the outcome is dependent on the particular **host** (genotype, age, previous exposures, immune status), the particular **pathogen** (strain, gene content, gene expression) and **host microbiota** (diversity of the microbiota and specific-species content)

Importance of host immune status

- Host genotype affects both innate and adaptive immune responses
    - Polymorphisms in immune genes (e.g. MHC, TLRs, cytokine genes, etc.)
- Immune response to pathogens is finely tuned between killing the pathogen and damaging the host
    - Appropriate immune response limits pathogen replication and spread, and therefore disease
    - Sometimes the immune response elicits host damage that gives clinical manifestation of disease (e.g. *Streptococcus pneumoniae* replicates in the lung but does not cause necrosis directly; instead it induces an intense inflammatory immune response that gives the clinical symptoms of pneumococcal pneumonia)

Importance of the host microbiota

- Diversity of the microbiota and specific-species content play an important role in the interaction between the host and pathogen, and determine different outcomes of disease
- The microbiota is critical for some gastrointestinal infections
    - E.g. *Clostridium difficile* infection usually follows antibiotic treatment which clears gut microbiota
    - E.g. *Citrobacter rodentium* (EPEC model) infections in mice; gut microbiota alters infection outcome by affecting pathogen gene expression and plays a role in host immune responses
        - HeJ and NIH mice have different gut microbiota
        - HeJ mice get lethal infections while for NIH mice there is usually no, or very limited mortality as a result of *C. rodentium* infections
        - Microbiota transplant changes susceptibility

What are pathogen virulence factors?

- Virulence is the relative capacity of a microbe to cause damage in the host
- Virulence factors are the factors which allow a pathogen to cause damage in a host, and cause disease
    - Virulence factor is the gene product produced by the pathogen (e.g. the protein, polysaccharide, etc.)
    - Virulence gene encodes the virulence factor
- Virulence factors are those that facilitate pathogenesis
    - Multiple virulence factors for a single pathogen
    - Expression of particular factors often dependent on host interaction
- **True virulence factors** are factors which:
    - **Cause host cell damage**, e.g. toxins (cholera, anthrax, botulinum, tetanus toxin)
    - **Facilitate colonisation** (i.e. colonisation factors to gain access), e.g. adhesins, pili, invasins, flagella
    - **Evade host immune system** (allowing persistence), e.g. polysaccharide capsules
- **Accessory virulence factors** are those that are involved in:
    - **Acquisition of nutrients,** especially those at low levels in the host (e.g. proteins for scavenging nutrients such as iron, amino acids and carbohydrates)
    - Secretion of virulence factors, i.e. **secretion systems** (may also secrete non-virulence factors)
    - Regulating expression of virulence factors, i.e. **regulators of virulence** (may also regulate non-virulence factors)
- But some virulence factors are difficult to define
    - E.g. acquisition of nutrients is common to pathogens and non-pathogens
    - The only way to determine if something is a virulence factor would be to test them experimentally (i.e. knock-out the gene of interest, and determine if the organism still cause disease in the host)
    - Also must consider that a virulence factor may work in one host but not another
- Housekeeping genes, such as general metabolic genes, are not considered virulence genes as they are found in all organisms and are not specifically associated with virulence

Why study virulence factors?

- Expression of virulence factors are intimately associated with disease
- Thus, understanding virulence factors gives us an insight into the disease process and so we may be able to stop the disease process
- Identification of virulence factors may identify drug targets and vaccine targets
- Many licensed vaccines are directed against virulence factors including:
    - Toxins e.g. diphtheria and tetanus vaccines use inactivated toxoid (symptoms in these organisms almost entirely due to the action of toxins)
    - Capsules e.g. *H. influenza* type b, *N. meningitidis*, *S. pneumoniae* vaccines are based on surface polysaccharide (survival of these organisms is due to capsule-mediated immune system evasion)

EXPERIMENTAL SYSTEMS

Choosing the right disease models

- Understanding virulence factors is highly desirable as an initial step in combating disease
- Need experimental systems for studying both the bacteria which causes the disease, and the bacterial/host interaction which defines the disease and the disease model itself

Picking the bacteria

- Where possible, study the organism which causes the disease

- However, often highly virulent strains are more difficult to work with
    - Sometimes very difficult to culture
    - Significant precautions to avoid disease in the people working with them
    - Often genetic systems are less well developed
- There may be multiple strains which cause different disease symptoms and disease severity

Picking a host/disease model

- In theory it is best to study the natural microbe/host interaction
    - But this is not usually possible for human diseases
    - Humans make reluctant subjects and are genetically variable
- So must find appropriate animal or cell culture model
    - Animals can be genetically defined and are cheap
    - However, may not show the same disease syndrome as humans
    - May not be affected by the same strains
- The perfect animal model should:
    - Display the same disease signs
    - Display similar tissue distribution of bacteria
    - Be acquired by the same route as the natural disease
    - Strains more virulent for humans should also be virulent in the animal model
- The above points are rarely all achieved, and the real test may be whether or not the model gives useful, testable insights into the real disease
- E.g. *Salmonella* Typhi which causes typhoid fever in humans is avirulent in mice, whilst *Salmonella* Typhimurium causes a mild non-systemic disease in humans and also causes a typhoid-like disease in mice
    - An advantage of using similar but not identical systems is that you will learn about similar diseases, but the disadvantage is that it is not the same disease (i.e. you will never be able to answer why *S*. Typhi specifically infects humans)
    - Recent advances in making 'humanised' mice may improve some infection models

Cell lines

- Advantages:
    - Reduces use of animals and therefore suffering
    - Less complex and better defined – can more easily test a specific hypothesis such as effect of a virulence factor on adherence, invasion, intracellular replication etc.
- Disadvantages:
    - They won't replicate the complete disease – results may not apply to the real disease which requires interaction of the whole animal and microbe

IDENTIFYING VIRULENCE FACTORS

There are two main approaches:

- **Biochemical approach** – identify a protein and determine its mode of action, and show that the action has a direct virulence effect
    - E.g. identify a toxin protein and show it has cytotoxic effects on eukaryotic cells
- **Molecular biology (genetic) approach** – identify a gene that when mutated affects virulence
    - Effect on virulence is only truly defined for the model/animal in which it is tested

Koch's Molecular Postulates

- The criteria necessary to prove that a suspected virulence gene results in a particular pathogenic phenotype (there is no universal agreement at present on what constitutes these postulates)

1. The virulence gene is always found in strains with a particular virulence phenotype

2. The gene should be expressed in the host during infection
3. Mutation (inactivation) of that particular gene reduces the virulence phenotype
4. Reintroduction of the gene reconstitutes the virulence phenotype

(KMP #3) How to mutate a gene?

- How can we mutate (inactivate) a particular gene that we think is a virulence factor to show that it is involved in virulence?
- There are two basic mutagenesis techniques:
    - **Random mutagenesis** (e.g. chemicals, radiation, transposons) – these methods inactivate genes randomly, and makes it hard to find which mutant has changed to the gene of interest
    - **Directed mutagenesis** – this involves making a change to only the specific gene of interest

Mutation of a particular gene

1. Use PCR to amplify the single gene of interest, and then clone the gene into a plasmid (small piece of DNA, few genes) that only replicates in *E. coli* (or the particular lab strain used)
2. Insert a new piece of DNA (usually an antibiotic resistance marker) into the gene of interest
    - This will do two things: it will make the new strain resistant to that particular antibiotic, and it disrupts the ORF of the gene of interest that we want to inactivate
3. While this does inactivate the gene of interest, how do we get the inactivated version back into the virulent bacterium's chromosome? Allelic exchange (recombination)
    - Transform the pathogen with the recombinant suicide plasmid (the plasmid will not be able to replicate in the pathogen)
    - Every cell expresses RecA which can carry out homologous recombination (though generally involved in repair of cells); and so in the pathogen, homologous recombination transfers the modified gene into the genome (cut, religation and transfer of similar sequences) thereby replacing the intact gene with the mutated version
    - The suicide plasmid cannot replicate and so is lost, and the new strain is a directed mutant and is antibiotic resistant (antibiotic resistance will select for strains where recombination has occurred)

How to measure effects on virulence?

- Must then test whether virulence has been impaired in the mutant
- Directly compare mutant with wild-type strain such as by:
    - Measure infective dose
    - Time to appearance of symptoms
    - Severity of symptoms
- Detailed analysis might show at what stage the gene is important (e.g. invasion, immune system evasion, toxin production etc.)
- Measurements are only informative if the animal model is an accurate representation of the real disease

(KMP #4) Complementing a mutant

- KMP #4 states that re-introduction of the gene should restore the virulence phenotype (complementation)—testing for this is important because it is possible that there may have been a mutation elsewhere and that the effects on virulence was due to this, or if the gene of interest is part of an operon, then the downstream genes may also have been affected
- Re-introduce the intact gene by cloning it into another plasmid that encodes another antibiotic resistance gene, and transform the mutant