MIIM30011 – MST1 – Full Lecture Notes – H1(93)

Contents

L6: Intracellular pathogens

Organisms can become adapted to replicate in a niche inside cells. Facultative organisms (eg. Salmonella) can live under **many different conditions**. Obligative bacteria cannot replicate outside of the host cell (much like viruses).

Intracellular replication can occur **within an endosome** or **in the cytoplasm,** which presents different metabolic challenges.

- There are advantages and disadvantages to each strategy:
    - Intracellular
        - **Advantages**
            - Hidden from innate immune system
                - Complement
                - Phagocytes
                - Antibacterial peptides
            - Hidden from adaptive immune system
                - Antibodies
            - No microbiome to compete with
        - **Disadvantages**
            - Very hostile unless adapted
                - In vacuole
                    - Nutritional requirements
                    - Limited by membrane
                - In cytoplasm
                    - Nutritional environment may be better
    - Extracellular
        - **Advantages**
            - Nutrients/substances more available
                - Klebsiella pneumoniae
        - **Disadvantages**
            - Exposed to innate defences
            - Have to compete with microbiome

After crossing the epithelial barrier, there are defence mechanisms that the organism must adapt to. It does so through the production of virulence determinants.

Intracellular Replication

The cell type into which a pathogen enters can influence its capacity to grow. Salmonella can grow in both **macrophages** and **epithelial** cells. In macrophages, there is only 1-5/infected cell, compared to 20-50 in epithelial cells, as the niche is more permissive to growth, and the epithelial is not antibacterial.

Cell Entry

There are two mechanisms of cell entry – ZIPPER and TRIGGER

Zipper – Receptor Mediated Endocytosis

- Exploits host cell pathways normally used for adhesion
    - Eg. Adhesion of epithelial cells to EC matrix
- Induced **receptor immobilization** and **clustering**
    - Induced by virulence determinants
- High affinity binding of bacterial ligand to host cell receptor
    - Endosome forms around bacteria, and it enters
- **Cytoskeleton dependent**
- Eg. Listeria monocytogenes

Trigger – Macropinocytosis

- Bacteria triggers own update by **injecting cell with protein effectors**
    - Through **injectisome**
- Cell **ruffling** induced by growth factors and hormones
- Brief contact by bacteria induces large-scale **actin polymerisation** and ruffle formation
- Ruffles fold over bacteria and engulf them
- **Cytoskeleton dependent**
- Eg. Salmonella enterica, Shigella spp.

Listeria monocytogenes - Zipper

- Soil organism
- GP rod
- Food borne pathogen
    - Unpasteurized cheeses and milk
- Causes CNS infections, maternofetal infections (infects baby through placenta)
    - May induce abortion
- 60-80 cases per year (rare), but high fatality rate
- **Enters via the zipper mechanism**
    - Internalisation requires **actin cytoskeleton** and bacterial protein internalin A (InlA)
        - Needs multiple interactions
            - Not just single engagement
- The cell receptors for bacterial invasins have other roles
    - E-cadherin **is the receptor** for the InlA 'ligand'
        - E-cadherin is attached to cell cytoskeleton and **forms tights junctions**
            - Joins 2 cells together
- 3 x InlA engages with 3 x E-cadherin
    - One engagement is not enough to rearrange the cytoskeleton
    - This **engagement causes clustering**
        - Endocytosis of the bacteria into a vacuole occurs

Salmonella – Trigger

- Salmonella invades the epithelium
  - Enters M cells and enterocytes
  - Undergoes transcytosis
- Invasion is **T3SS mediated,** with a number of **effector proteins.**
- Membrane ruffling is induced by the injection of these effectors via a T3SS injectisome
  - Salmonella has 2 x T3SSs
    - SPI-1 (Salmonella Pathogenicity Island-1) T3SS
      - Needed for initial invasion
    - SPI-2 T3SS
      - Needed for survival in macrophages
        - **Modifies the vacuole** to stop lysosomes fusing

PAIs were discovered when the DNA was sequenced, to find that the virulence determinants for a pathogen were linked together – now known as a PAI. 2 of them encode T3SSs in Salmonella.

- There are a few steps to Salmonella infection, involving 50+ effector proteins
  - Entry to establish the replicative niche
    - The **replicative niche is the Salmonella containing vacuole (SCV)**
    - T3SS-1 (encoded by SPI-1) triggers entry
    - Many proteins injected that helps the organism enter
      - Many functions such as blocking components of cell function
        - Some effectors have **multiple roles** which can effect more than one host cell protein
          - Sorting base sorts what proteins are injected
    - **Proteins induce ruffling** and the Salmonella is brought up in a vacuole
  - Modify the SCV to protect against host cell responses
    - Different effectors now injected
      - Help early SCV develop
    - The bacteria needs to **avoid lysosomal fusion and apoptosis**
      - Salmonella **blocks early apoptosis** to keep the cell alive
        - The cell senses the Salmonella proteins in the cytoplasm
        - Tries to die
    - The vacuole matures and changes shape as it does
      - T3SS-2 (encoded by SPI-2) **involved after the SCV matures** and **produces SIFs**
        - Secretes proteins which get the SCV to grow
        - Produces filaments (SIFs) that allow the volume to increase
          - The SCV changes shape as it matures.
          - Once mature, T3SS-2 expressed which ejects diff proteins
            - Gets the SCV to grow and produce filaments
              - SIFs allow the volume to increase
    - The cell will ultimately die when full of bacteria

In summary:

- Create a niche (SCV) after trigger entry
- Modify the SCV

- Replicate a lot (epithelium) or a little (macrophage)
- 100+ effectors
    - Alter normal cell biology
- Avoid killing/destroying niche, modifying membrane SCV for nutrients including SIF formation

Leaving the Vacuole

Once bacterium enter the cell, they can choose to stay in the vacuole or leave the vacuole.

- Stay in vacuole
    - **Lysosome-fused vacuole**
        - Intralysosomal
        - Low pH
        - Hydrolytic
            - Eg. Coxiella spp
    - **Entry vacuole**
        - Neutral pH
        - Intravacuolar
        - **Block fusion of lysosome** to allow permissive niche
            - Eg. Salmonella, Legionella
- Leave vacuole
    - **Become cytosolic**
        - Eg. Listeria, Shigella

An unfused cell vacuole (eg. Salmonella (SCV), Legionella (LCV)) separates the bacterium from antibacterial processes (such as lysosomes). However, some bacteria leave the vacuole. **This is not dictated by the entry process.**

- Listeria monocytogenes
    - Produces Listeriolysin O
        - Phospholipase
        - Breaks down the vacuole membrane
- Shigella spp
    - Have a **contact hemolysin**
        - Degrades the vacuole upon contact with the membrane

Both can move through the cytoplasm using a polar protein which polymerises actin, found at only one pole of the bacterium. They want to do this as **if they stay in one place in the cytoplasm, nutrients can become depleted.**

Shigella spp

- E.coli related, GN rods, GE and dysentery causing esp in early life in developing world
    - S. dysentariae, flexneri etc
- Movement is driven by bacterial protein IcsA
    - IcsA is an **autotransporter**
    - Only **expressed at one pole**
        - IcsA – Intracellular Spread

- Under immunofluorescence, the bacteria is red and the filamentous actin is green.
    - The yellow protein on the end is IcsA
- IcsA engages with a **cell cytoplasm protein** called N-WASP
    - IcsA has many **glycine rich repeat regions (GRRs)** which make contact with N-WASP
- IcsA expressed at pole of bacteria
    - N-WASP recruited and activated
        - When **inactive, N-WASP is folded**
        - **When it binds, it unfolds** and recruits Arp2/3 complex
            - This then recruits g-Actin (soluble) which is used to form filaments of F-actin (filamentous actin)
                - The actin is recruited and then **polymerised.**
                - Generation of the filament pushes the bacteria forward

Listeria

- Listeria has ActA which causes **filamentation of actin** on one end
    - ActA drives this filamentation
- After entry via the zipper mechanism, Listeriolysin O **lyses the membrane**
- The bacteria proliferates in the cytoplasm, then produce ActA
- Polymerization of actin drives movement around the cell, and sometimes into **adjacent cells**
    - If it enters another cell, it will be in an endosome and will again use Listeriolysin O to lyse the membrane
        - Through this process the bacteria does not leave the cell, and so is not exposed to phagocytes, complement etc

Summary of pathogenesis lectures

- Bacteria arrive from somewhere
    - Microbiome shifted (inside the body)
    - External (infectious)
- Bacteria colonise using protein structures to overcome repulsive forces
    - Pili, Fimbriae (eg. Mrk)
- Grow on/in epithelium
    - Stay local (eg. cholera)
    - Or spread systemically
        - If systemic, can be EC or IC
- Need to avoid innate defences
    - Complement, antibacterial peptides
        - If EC, need to avoid phagocytosis
            - Eg. use a capsule, leukotoxins
        - If IC, growth rate depends on the cell type
            - IC growth has led to the evolution of complex biology
                - Making and sustaining a niche is hard
                    - LLO, ActA for cell spread

L7: Bacterial protein expression

Bacterial infections may not cause any damage, which will lead to asymptomatic infection.

Damage from bacterial infection may come from:

- **Exotoxins**
    - Drive the most serious consequences of infection
- **Endotoxins**
    - **Part of structure** of organism
        - Eg. LPS
    - Toxins bind to specific receptors
        - The receptor have evolved for other functions
- **The host**
    - Much of the damage comes from the host response
        - Complement activation
        - Cytokine release
        - Toxins altering normal physiology
        - Pus
            - All alter normal physiology – signs and symptoms of disease

Bacterial Exotoxins

Exotoxins are heat labile, protein, toxic substances that are released by some bacteria into the surrounding. Exotoxins are among the most lethal and toxic substances known. They are toxic even in nanogram-per-kilogram concentrations.

- **Proteins,** often regulated
    - Made at certain parts of infection
        - Not always constitutively
- Some have common but not universal features
    - **ADP ribosylation**
        - When an ADP-ribose group is transferred to a carbohydrate
        - Common feature of many exotoxins
            - Conservative evolution
- **Toxic** at very low levels – ng/kg
- Usually have 2 domains
    - **A – enzymatic (active) domain**
    - **B – binding domain**
        - Sometimes a single protein (eg. tetanus toxin)
            - May come from different proteins and then associate together
- Some of the toxins are **encoded by a bacteriophage.**
    - Understanding where they come from is not fully resolved.

<u>Changes that impact pathogenesis:</u>

- **Normal flora can migrate**
    - Are non-pathogenic in the normal site
    - But, for example, E. coli in UGT can cause UTI

- Pathogens have **acquired virulence determinants as additional genes**
    - Normal genome + extra DNA for VDs
    - These are **not mutations,** though mutations may occur to modify the determinants to increase activity
        - A normal flora could come into pathogen and **become transformed** through lysogeny, for example
            - The genes can come from **horizontal gene transfer**
                - Acquire viruses
                - Acquire plasmids
                - Acquire genes
                    - Through processes like transformation, conjugation, transduction

Mutations

- Mutations happen at a **low level**
    - **DNA polymerases** make mutations during **DNA synthesis,** at a low rate
        - The mutation rate in eukaryotes is low
        - The mutation rate in RNA viruses is high
            - Bacteria outlier group of H. pylori causes stomach cancer
                - This organism has a **highly plastic** genome
                - Has a **high mutation rate** that is generally much different to other bacteria

The rate of mutation in bacteria like H. pylori is high but **not high enough to transform a bacterium from non-pathogen to pathogen.** It would have to **acquire DNA** to do so.

SNPs

When a genome changes through mutation created by DNA polymerase, this SNP may be synonymous (in that it alters the AA it encodes) or non-synonymous.

- The probability of getting an AA change is higher if the change is in position 1 or 2
    - It may not change the AA, but this does not necessarily mean that the RNA will not change
        - May get a **rare codon** which affects **translational efficiency**
            - Need to find a tRNA to integrate
            - This **may affect the protein level**
                - tRNA may be rare
- SNPs may also **change the structure of RNA**
    - RNA is highly structured
        - Can base pair on itself
        - This can **affect RNA activity**

Genome position

When genes move or integrate in the chromosome, **position affects the protein dosage during bacterial replication.** Insertions **close to the ori** duplicate early in the replication cycle, meaning it may **more efficiently produce protein.** This is because it **spends more time as a duplex** rather than a single copy.

Bacteria are highly efficient and optimised:

- Bacteria have been evolving for billions of years and are therefore efficient and optimised
    - They have evolved to occupy a certain niche
        - Have a reduced genome to be the right size for that niche
            - **As bacteria become more obligate, genome size decreases**
            - **Host adaptation results in gene loss.**

To transform from normal flora to pathogens/acquire resistance, mutation rate is too low to create this. More rapid changes can occur from HGT acquired from other bacteria and viruses.

Acquisition can change behaviour of the pathogen.

Pathogenicity islands

- Often **vestigial bacteriophages**
    - Started off as an integrated bacteriophage
    - Through replication, they lost capacity to leave
        - The phage has **become fixed**
            - May have lost excision genes etc
            - **No virus particles made**
                - Many genes encoded still functional
- Insert into **phage insertion sites**
    - Seen in sites such as **tRNA genes**
        - These are **redundant** and the bacteria don't tend to use them
        - Specially targeted by a bacteriophage
- Have **different %GC contents**
    - Seen in genome analysis
    - May go up or down depending on where the DNA has come from
- Includes:
    - Cholera toxin phage
        - **CTX,** Vibrio cholerae
        - Without phage, doesn't cause cholera
            - The phage is still **competent – can come and go**
    - Corynebacterium diphtheriae
        - **Beta prophage**
            - The absence of phage means the bacteria is non-toxigenic

Salmonella has 2 PAIs – SPI-1 and SPI-2 but other bacteria may have more.