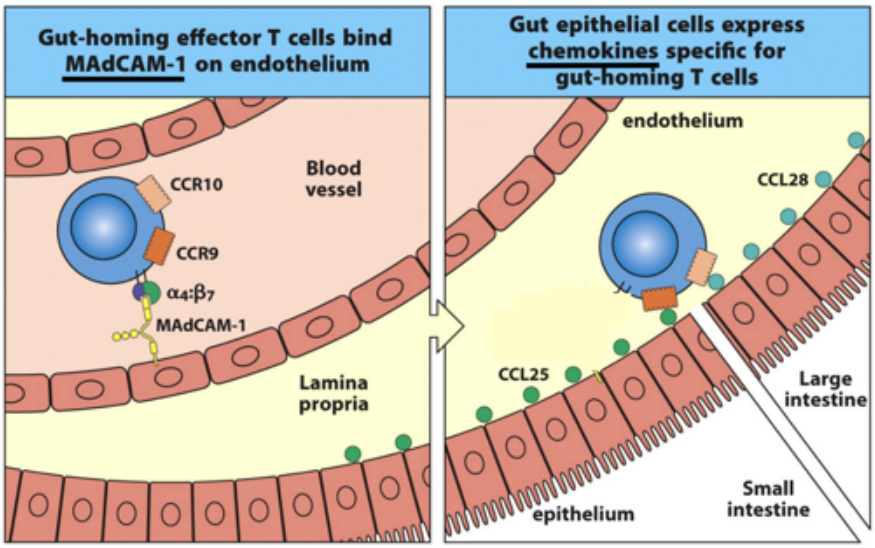
Microbes, Infections and Responses

SEMESTER 2



- The mucosal addressin that they are induced to express when they are activated (2nd part of image above this) are known as α_4: β_7 integrin.
    - + Only the cells activated in the PP or MLN will express this integrin.
        - So, lymphocyte activated in the peripheral lymph nodes (spleen, lymph nodes etc) will express different addressins.
    - + It also induces Chemokine Receptor 10 (CCR10) and Chemokine Receptor 9 (CCR9) expression.

- The α_4: β_7 integrin expressed on mucosal activated lymphocytes is a ligand for a molecule known as MAdCAM-1 (Mucosa Addressin Cell Associated Molecule) selectively expressed on endothelial cells which line the HEV at mucosal sites only.
    - + Then, they wait for a second signal (chemokines) produced by the gut epithelial cells of small (CCL25 → Bind to CCR9) and large (CCL28 → Bind to CCR10) intestine.

- In summary, lymphocytes circulate around our body → recognise MAdCAM-1 expressed on HEV of mucosal sites **only** and Bind. Then, they wait and respond to the chemokines produced by gut epithelial cells.
    - + Since, MAdCAM-1 is expressed on many mucosal tissues; the immune activation at one mucosal site, may provide an effector response at other mucosal sites (including breast tissue).

Antibody Production

- B cells encounter antigen in PP (gut lumen antigens enters by M cells)
- APCs + T cells: Provide appropriate signals for B cell help → B cell proliferation and IgA production (TGFβ)
- After activation in gut lymphoid tissue, lymphocytes acquire homing receptors ($\alpha_4\beta_7$), and chemokine receptors specific for lamina propria chemokines.
    - + Activated IgA producing B cells can leave the inductive sites and migrate to effector sites via blood.
        - Most antibodies produced by B cells activated in mucosal sites produce IgA.
        - B cells also use ($\alpha_4\beta_7$) and chemokine receptors in the same way as the T-cells.
    - + Bind epithelium of lamina propria and secrete IgA into mucosal lumen.

- IgA differs in mucosal secretions and blood:
    - + IgA is produced and secreted as a dimer linked by joining chain at lamina propria; however, it circulates as a monomer.
    - + It is transported to mucous secretions: IgA is associated with another protein referred to as the secretory component.

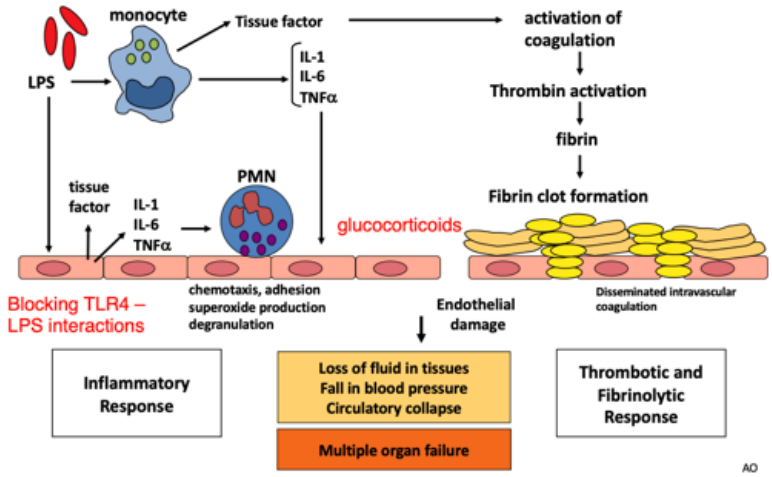
- IgM in secretions as pentamer (linked by J-chain as well).

(1) - Using PRR Agonists to Block PRR Signalling and Suppress Immune Responses

- Where at times we want to stimulate inflammatory reactions and deliberately ligate PRRs to induce a wanted outcome, we must also consider the use of antagonists of PRR to suppress immune responses:

    + Blocking Over-Exuberant Immune Responses:
        - TLR-4 antagonists in trial for septic shock.
    + Deviating Immune Responses:
        - TLR ligands as immunotherapy for allergies.
            - Trials using TLR-2 agonists suggest clinical improvement in allergic rhinitis patients, if they counter excess T_H2 cytokines that promote IgE and eosinophil recruitment.
            - So, can we deliberately engage with TLR ligands to change the T_H2 type responses to that allergen and drive it towards T_H1 type responses?
                - We are not trying to induce inflammation but to reduce the allergic reaction.

Septic Shock



- Septic Shock is another example of where we try to interfere with strong inflammatory reaction.

    + Septic shock is often the result of the presence of large amount of replicating bacteria in the bloodstream → Can happen with both, gram+ve/-ve bacteria.

- In the context of a gram-negative infection, there are large amounts of LPS present due to replicating/dying bacteria → LPS will engage with TLR-4 expressed on blood cells (e.g. monocytes) and endothelial surfaces.

    + This results in the release of inflammatory cytokines (IL-1, IL-6 and TNFα) → Activate endothelial cells → Increase in permeability and neutrophil migration → Neutrophil activation → Produce superoxide chemicals → Degranulation.

- What happens in septic shock?

    + When monocytes are activated, they also produce Tissue factor (+ inflammatory cytokines) → Activation of blood coagulation cascade.
        - LPS producing widespread inflammatory response, but also activates blood coagulation.
        - Loose endothelial cells allow for the deposition of small fibrin clots everywhere along the blood vessels (Disseminated intravascular coagulation)
          → Thrombotic and Fibrinolytic Response.
    + This ultimately results in damaged endothelial cells that line the blood vessels:
        - Loss of fluid in tissues, Fall in blood pressure, Circulatory Collapse → Multiple organ failure.

- So, we try to use TLR4 antagonists to interfere with these processes → Varying success because once that cascade starts, it is very difficult to control it and reverse it
- We can also use glucocorticoids to decrease the movement of neutrophils and monocytes from blood vessel into the tissue.

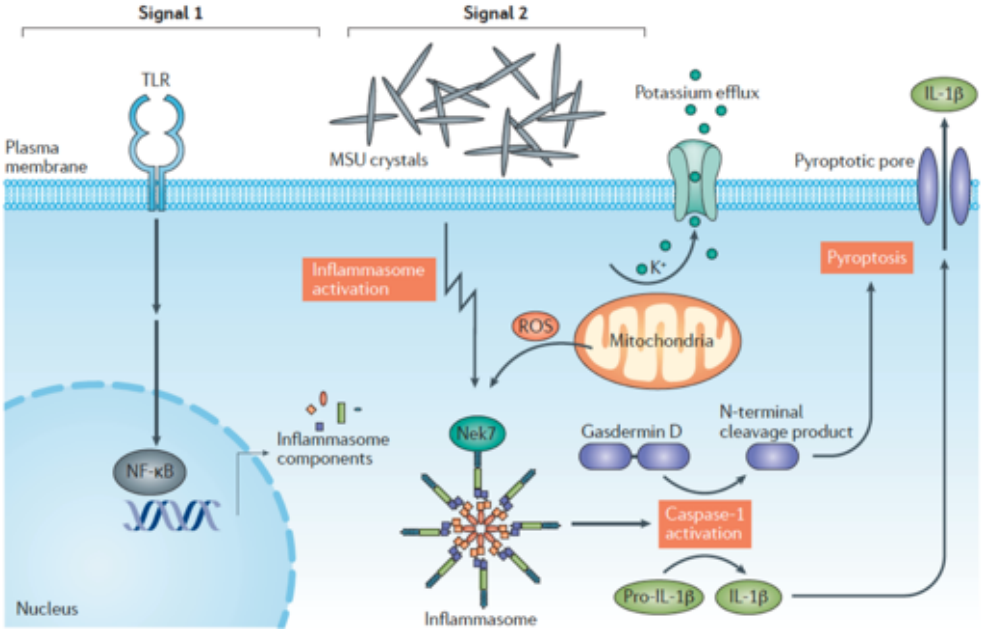
(2) Manipulating Cytokines to Boost Induction of, to shape or to Suppress Immune Responses

- Boosting APC activity:
    + IL-12 therapy to promote T_H1 response important for intracellular pathogens
        - E.g. TB under consideration.

- Boosting Antiviral Responses:
    + Administration of Type 1 interferons (IFNα, IFNβ); E.g. in hepatitis C virus (HCV) infection.

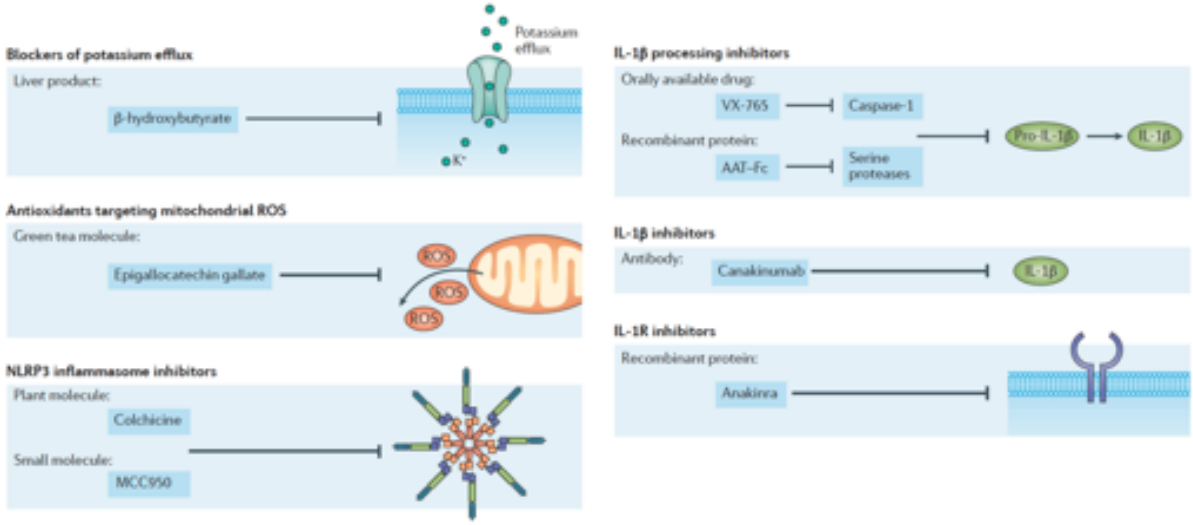
- Antibodies against inflammatory cytokines or receptors for inflammatory cytokines:
    + To control inflammatory conditions – Abs to IL-1 or IL-1R for gout, Type 2 diabetes, autoinflammatory disorders or Abs to TNFα or TNFR for rheumatoid arthritis.

Gout – Uric Acid Crystals and Inflammation



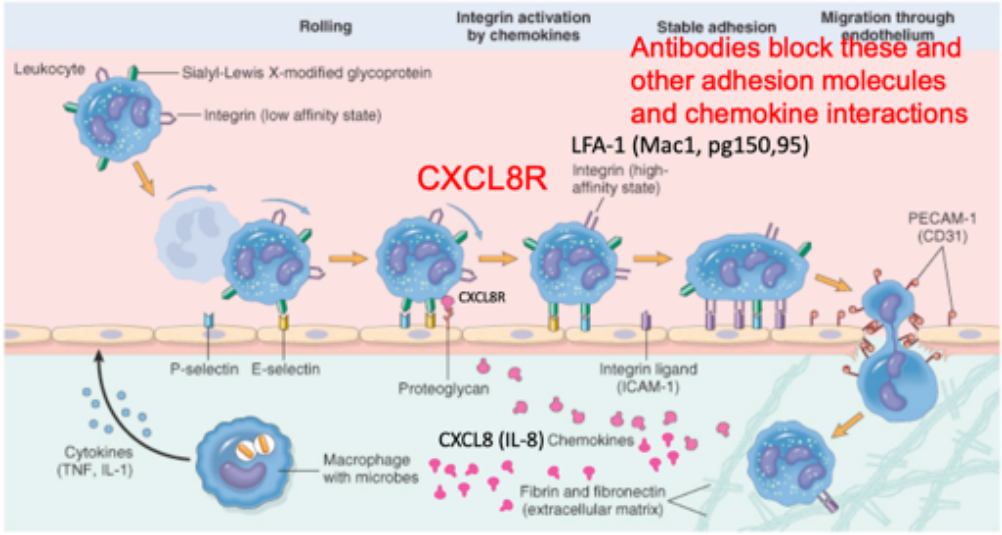
- Gout is due to an inflammatory reaction in the joints:
    + The inflammatory reaction is mediated by an innate immune response to Uric Acid Crystals.
        - It's **not** an inflammatory reaction caused by an infection; It's an inflammatory response induced in response to **DAMP** (not PAMP).
        - Uric acid crystals (MSU) come from metabolism due to high cholesterol intake.
    + It is not only the uric acid crystals that drive this inflammatory reaction.
        - We know from experiments that there are **2 signals** involved.

- The deposits of uric acid crystals are *driving* the inflammatory reactions → Interaction with inflammasome complex (signal 2)
    + Inflammasomes are complexes that form in the cytosol in response to certain signals from PRR like TLR to drive the first assembly of inflammasome → Signal 1.
        - We **don't** know what the trigger was for the formation of inflammasomes → Signal 1 missing.
    + Interaction of MSU with inflammasome in innate immune cells triggers the production and release of certain inflammatory cytokines.
        - Result in signalling cascade 2 → Activation of Caspase-1 → Activation of pro-cytokines (pro-IL-1β→ IL-1β and pro-IL-18 → IL-18) → Release from cells.
            - IL-1β drives the strong inflammatory reaction → Activation of endothelial cells and synovious sites present in the joints (these cells have receptor for these cytokines) → Release of more cytokines and chemokines from these cells enhance inflammatory reactions and recruitment of neutrophils.



- One way of thinking about therapy for gout (→ suppressing inflammatory reactions):
    + It is very hard to remove the uric acid crystals, but you can reduce it through a change in your diet.
- We **don't** know the 1st signal that stimulated these inflammatory reactions, so much of the therapy focus on the outcomes of the activation of signalling cascades (shown above).
    + I.e. We are not removing the cause of gout but interfering with the outcome and mediators of the response.

Macrophage and Complement Components Induce Neutrophil Migration from Blood to Tissues

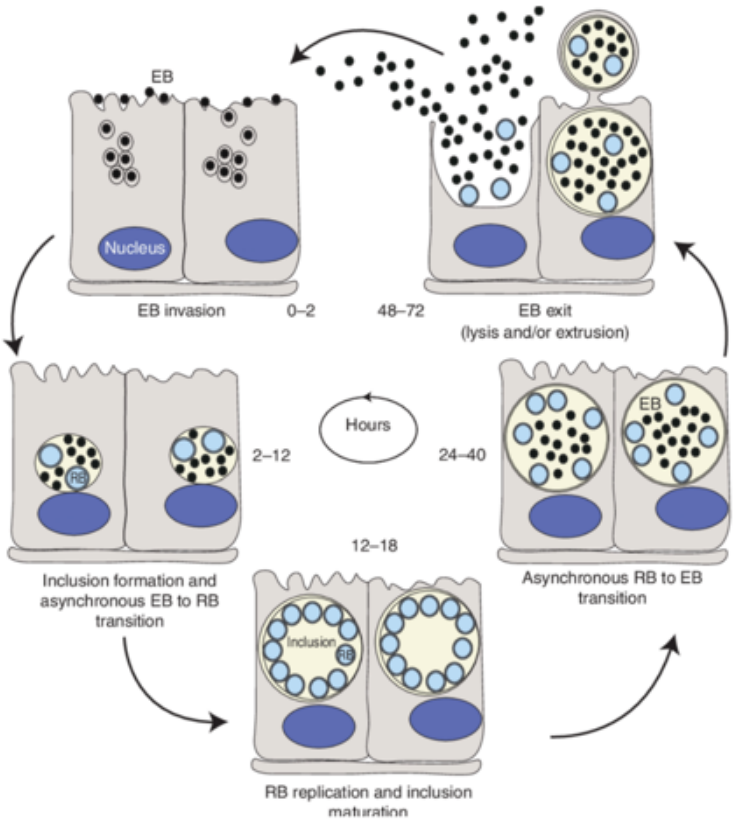


- We can also interfere with recruitment of neutrophils from blood to tissues:
    + Macrophages release cytokines that act on endothelial cells → Adhesion of neutrophils to these endothelial cells.
    + Chemokines (IL-8 or CXCL8) produced by macrophages are also presented on the surface of endothelial cells, and neutrophils with CXCL8R can recognise them.
    + Neutrophils also use molecules like LFA-1 (Mac1, pg150,95) and other molecules to adhere to these endothelial cells and ultimately migrate through endothelium to the site of infection.
- Using antibodies to block these adhesion molecules and chemokine interactions is one way of interfering with recruitment of neutrophils.

Chlamydiae: Unusual Bacterial Genus

- **Gram Negative** – Outer membrane LPS is truncated and not very endotoxic (weak activity)
    - **No peptidoglycan** → Not detected by Gram staining.
        - You will see lymphocyte response but no bacteria in Gram stain of genital discharge.
- Small (0.25 – 0.8µ)
- **Obligate Intracellular Bacteria** → Energy Parasite: Need host cell for energy to survive.
- Unique replication cycle (2-3 days): **Two Developmental Stages**:
    - Elementary Body (EB) → Hardy and survives outside of host cell.
    - Reticulate Body (RB) → Vulnerable but replicates inside host cells.
- Damage caused predominantly by the host immune response (chronic inflammation).

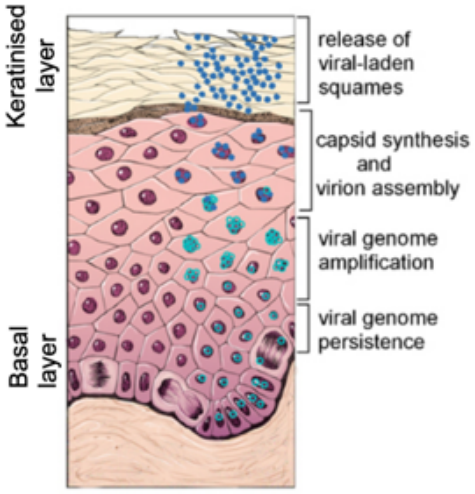
Chlamydiae Lifecycle:



1. EB of chlamydia binds to columnar epithelial cells → Taken up through receptor mediated endocytosis.
    - In immune cells, endosomes would fuse with lysosome to kill the bacteria **but** doesn't happen in columnar epithelial cells with chlamydia.
2. Multiple endosomes will gather together and form an Inclusion body with EB inside.
    - EBs convert to RBs asynchronously → Mixture of EB and RB in the same inclusion.
    - RBs will then replicate inside the inclusion but aren't released as they can't survive outside the cell.
        - RBs will revert back to EB asynchronously.
3. The EB is exported out of the cell through reverse process of RME or cell lysis.
    - Released EB can infect other cells of the host or infect cells of new person (if there is contact).

- After they have been endocytosed, they are isolated from the nutrients → They stick drinking straws out of inclusion bodies and into the cytoplasm to obtain nutrients and energy.

Encounter and Entry



- HPV particles on surface of warts and can be spread by **direct** skin-to-skin contact (no viruses in blood)
    - + Skin is highly resistant to infection, but virus can enter through scratches and target the basal cells.
- Infections **more** likely when virus comes in contact with mucous membranes:
    - + Keratinous layer of mucosal membrane is thinner than normal skin but still needs microabrasions.
        - These occur with vaginal, anal or oral sex and moist mucosal epithelia promotes efficient spread to the penis, anus, vulva, vagina, cervix and less frequently, oropharynx and larynx.
- Replication cycle:
    1. HPV inserts its genome (circular episome) into the basal cells and don't replicate immediately.
    2. It produces E2, which links the viral episome to DNA in mitotic chromosomes on the spindle ensuring partitioning of the episome.
        - E6/E7 are expressed at this early stage and they bind to cellular proteins that control cell division → Promote cell replication.
            - Probably responsible for the benign proliferation in warts.
    3. As the cells mature and move towards skin surface, the episome is also transported to upper layers.
        - Here, other early viral genes are expressed, and viral DNA replication begins.
    4. Capsid proteins L1/L2 (and E4) are only made in cells that have differentiated into keratinocytes.
        - These form the non-replicating barrier layer of the skin.
    5. Viral DNA is packed into capsids to form progeny virions in the keratinocytes nucleus and are shed with these cells at the skin surface.

Immune Response

- There is poor immune response to PV because:
    - + Virus only shed from skin surface.
    - + No viral antigen secreted from cells.
    - + Virus not blood borne.
    - + Infection does not result in dead cells.
- DC and Lehninger cells are also found in skin but not in the edges of keratinised layer → APCs may not come across these rare viral antigens presented:

    → Expect little or no viral antigen to travel to lymph nodes.
    → Expect little or no antigen to be taken up by DC in the skin.
    → Expect little or no inflammatory response to activate the DC to migrate to the lymph nodes.
- Hence, poor immune response induction → Lack of viral clearance.

L29: Legionella

Legionnaire's Disease

- Acute form of pneumonia
- "Pontiac fever": Self-limiting flu-like illness.
- ~50 species of Legionella
- >90% cases caused by Legionella pneumophila (serogroup 1)
- In Australia, approximately **50%** of cases are caused by Legionella longbeachae
- An emerging pathogen.
- **Gram-negative** environmental (soil/water) organism → intracellular pathogen.
- When people inhale aerosol containing this organism, the symptoms of this disease start to appear usually within a 2-10-day timeframe.
    - The symptoms are non-specific – people would just feel crappy, cough, fever, muscle ache, and a decent proportion of Legionella infections are misdiagnosed as something like cold or flu.
- One of the biggest risk factors for contracting Legionella is age → Older people are more at risk due to decline in immune system function.
    - Also, males are much more likely to get infected than women.
- There's about 50:50 ratio of infection with L. pneumophilia or longbeachae.
- There are many Legionnaires' disease outbreaks.

Transmission

- Legionnaire disease:
    - Can be community acquired or nosocomial.
    - Due to inhalation of aerosols.
    - **No** person-to-person spread.
    - Risk factors include immune deficiency, age, smoking, drinking and male gender.
        - Many of these are those that impact the function of lungs.
- Legionella pneumophila is associated with man-made water sources (cooling towers, spas, fountains, ventilators, birthing pools, car wash, showers, hot springs etc.).
- Legionella longbeachae is associated with soil environments, exposure via inhalation of contaminated potting mix (→ Masks can protect from contracting this disease).
    - Potting mix in Australia has legionnaire's disease as one of the risks.

Legionella Infection

- When an individual is infected, as long as the individual is diagnosed within a reasonable period of time, treatment of antibiotics is very effective.
    - There's very little antibiotics resistance.
        - If you think about the environment and selective pressure on Legionella, you can understand why.
        - It exists in water and not transmitted from human to human → It's contact with antibiotics is minimal → Unlikely to develop antibiotic resistance.
- Erythromycin (newer macrolides) or quinolones are used for treatment.
- Urinary Antigen Test is commonly used for diagnosis → Rapid diagnostic test.
- Like with most bacterial infections, the gold standard is culturing the bacteria from sputum.
    - Grows on charcoal Agar
        - Charcoal helps to reduce oxidation of cysteine and detoxifies fatty acids produced from replication.
    - Requires high levels of cysteine and iron for growth.