Case 3 – The Immune Response

(Ashley) Hee Seung, Kim

From India to Canada

53-year-old Robert K. immigrated from India about a year ago. Over the past month he has had fevers, chills, night sweats and a chronic productive cough. He goes to see his family doctor who confirms a fever of 38.5°C. Upon auscultation she also finds crackles in the right lung and decreased breath sounds in the right lower lung field. She sends Robert for a chest X-ray and gives him three sterile containers with instructions to generate three deep sputum samples over three mornings. After the samples are examined in the Microbiology Laboratory the Public Health Unit notifies Robert K. to report to the local hospital for further assessment.

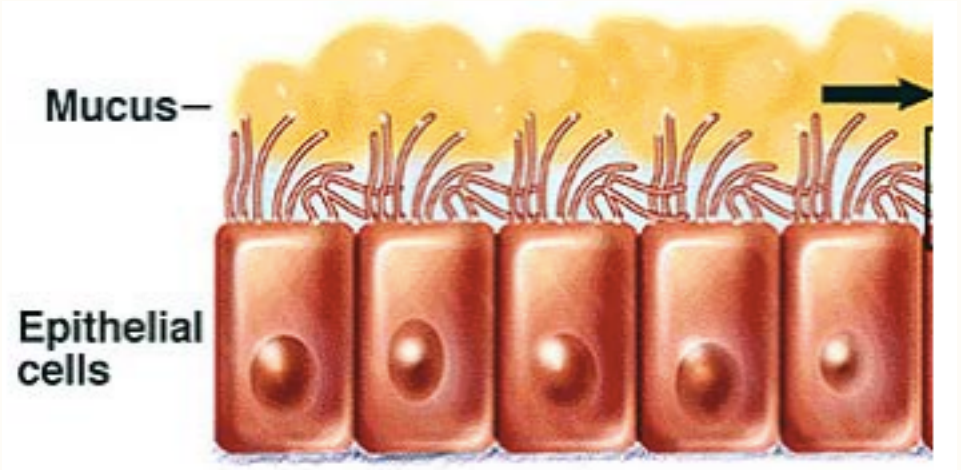
Question 1 – Host Response

What elements of the innate and adaptive (humoral and cellular) immune response are involved in this infection?

Three lines of defense

1. Anatomical & Physical Barriers

- Skin
- Mucous membranes
- Low pH of the stomach
- Body temperatures



For case 3, mucociliary transport system is the most important

- Mucus secreted by goblet cells trap pathogens
- Motile cilia moves it up the airway, allowing pathogen containing mucus to be swallowed and killed by stomach's acidic pH

2. Innate Responses

Innate response is a non-specific response towards general pathogen species involving:

- Phagocytosis
- Opsonization
- Activation of the complement cascade
- Chemotaxis of phagocytic cells
- Activation of the Inflammatory response

Innate response has two parts: the immediate response and the induced response

2. Innate Responses

Mucous

Lysozyme	degrade glycosidic bonds of bacterial peptidoglycan
Secretory IgA	recognize receptor binding domains on pathogens, neuralize, and prevent them from binding to mucosal cells
Lactoferrin	sequester iron from pathogens (iron is necessary for pathogens' actions)
Lactoperoxidase	enzyme that generates toxic superoxide radicals causing death of pathogens

2. Innate Responses

- Surfactant proteins SP-A and SP-D
  - Block bacterial surface components, promote phagocytosis
- Defensins alpha and beta
  - Active against gram-positive bacteria like *S. pneumoniae* and *M. tuberculosis* to form a pore complex in bacterial membranes causing a membrane depolarization and lysis
- Cathelicidin LL-37 (CAMP)
  - Inserted into bacterial membranes causing lysis

2. Innate Responses - PRRs

- When host mucosal and epithelial layers are breached:
- **Pathogen recognition receptors (PRRs):**
  - Recognize bacterial pathogens by their pathogen associated molecular patterns (PAMPs)
  - PAMPs include lipopolysaccharide (LPS), mannose bacterial DNA or RNA, peptidoglycans and lipoteichoic acids, lipoproteins, flagellin, and microtubules

1. Phagocytosis receptors (on macrophages, neutrophils, and dendritic cells)

- PAMP recognition induces phagocytosis; endosomes fuse with lysosomes and pathogens are degraded by reactive oxygen species or reactive nitrogen species

2. Innate Responses - PRRs

2. Toll like receptors

- PAMP recognition induces internal signaling cascade leading to cytokine release
- TLR2 binds poptidoglycans and lipoproteins of gram-positive bacteria like *S. pneumoniae* and *M. tuberculosis,* respectively
- TLR4 binds pneumolysin, which is released by *S. pneumoniae*, causing pathogen apoptosis
- TLRs activate NF-kB, which leads to

  -> production and secretion of Type 1 interferons (alpha & beta) that lead to

  -> activation of NK cells that activate

  -> macrophages, T cells, DC cells secrete cytokines that cause

  -> inflammatory response

2. Innate Responses - PRRs

3. C-type lectin receptors

- Recognize mycobacterial glycolipid trehalose dimycolate to trigger cytokine signaling response

4. NOD-like receptors

- Sense muramyl dipeptide of *S. pneumoniae* and *M. tuberculosis*

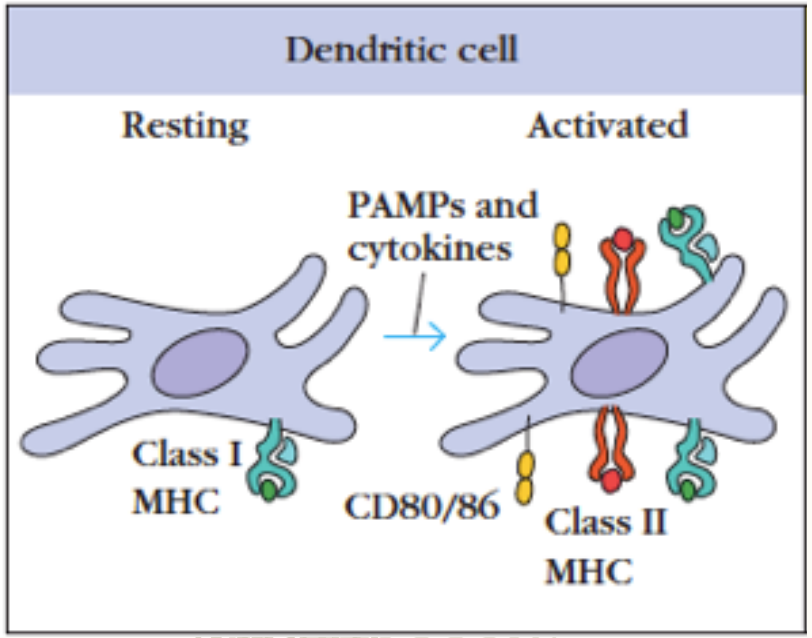
5. RIG-1-like receptors

- Sense viral replication by interacting with dsRNA of pathogens

2. Innate Responses

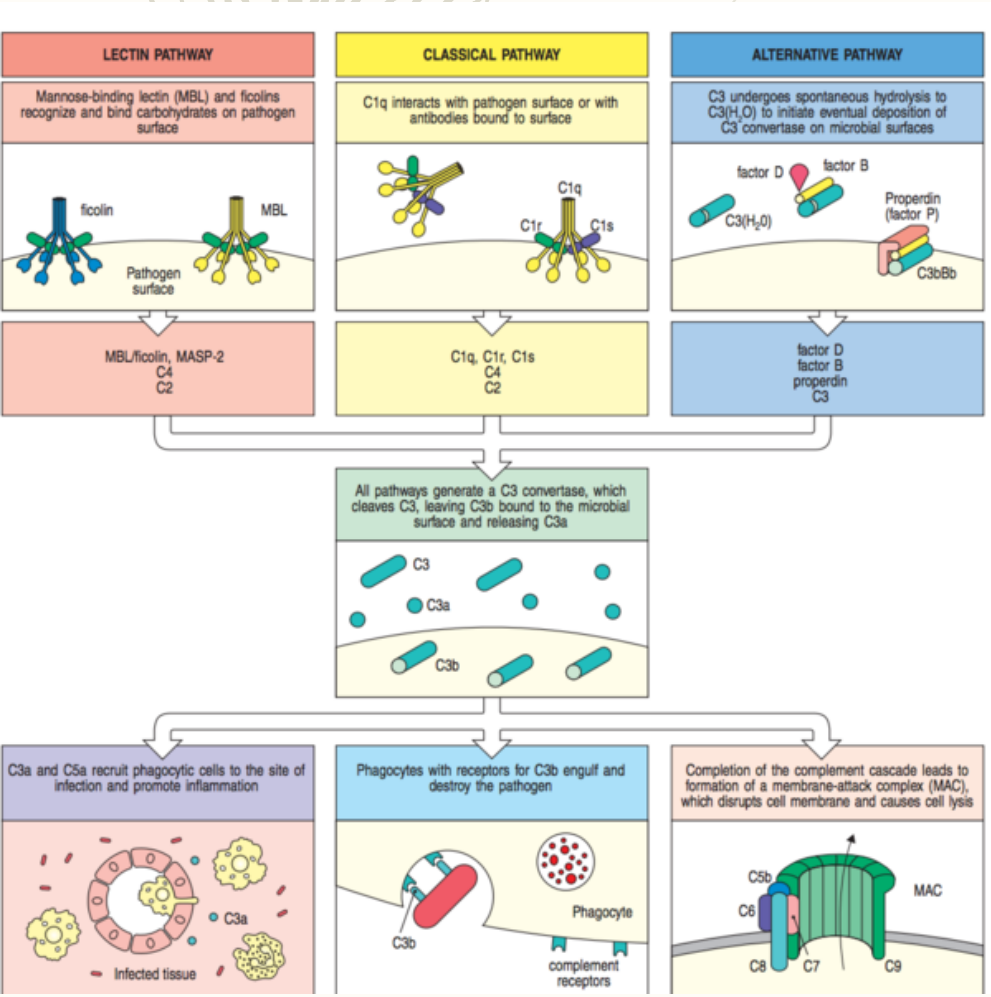
- Tumor necrosis factor alpha
  - Activates macrophages
  - Increases vascular permeability of epithelium, resulting accumulation of fluids into infection site
- TNF: transmembrane protein
  - Binds to TNFR1 and TNFR2 to induce anti-apoptotic, pro-inflammatory signals
- TNR1: induce apoptotic and anti-inflammatory response
- Neutrophils
  - Recruited by chemokines and cytokines
  - Move to the infected site via diapedesis and kill bacteria via phagocytosis, degranulation, and neutrophil extracellular traps
  - Neutrophils capture *S. pneumoniae* and degrade it using myeloperoxidase antimicrobial enzymes

Transition to Adaptive Responses – Dendritic Cells



- It is initiated by **Dendritic Cells (DCs)**
- DCs PPRs recognize bacterial PAMPs and become mature DCs
- Mature DCs have higher levels of MHC and granule antigens on their surface, the production of cytokines, and the expression of key co-stimulatory molecules CD80 and CD86
- MHC II on DCs present antigenic peptides to T-cell Receptor (TCR), leading to IL-2 transcription
- Co-stimulatory B7 of DCs also binds to CD28 of naïve T cells to generate IL-2 secretion
- DCs activation of naïve T cells into mature cytotoxic T cells or helper T cells is important in adaptive responses

Transition to Adaptive Responses – The Complement System



Complement proteins are activated via one of the three complement pathways:

1. The Classical Pathway

- initiated by IgM or IgG binding to a multivalent antigen to generate C3 convertase

2. The Lectin Pathway

- Mannose Binding Lectin (MBL) and ficollins bind to PAMPs (surface carbohydrates) to generate C3 convertase

3. The Alternative Pathway

- initiated when C3 (third component of the complement) undergoes a specific breakdown, forming C3b that adheres to a cell surface

* The formation of C5 convertase is the same for all pathways

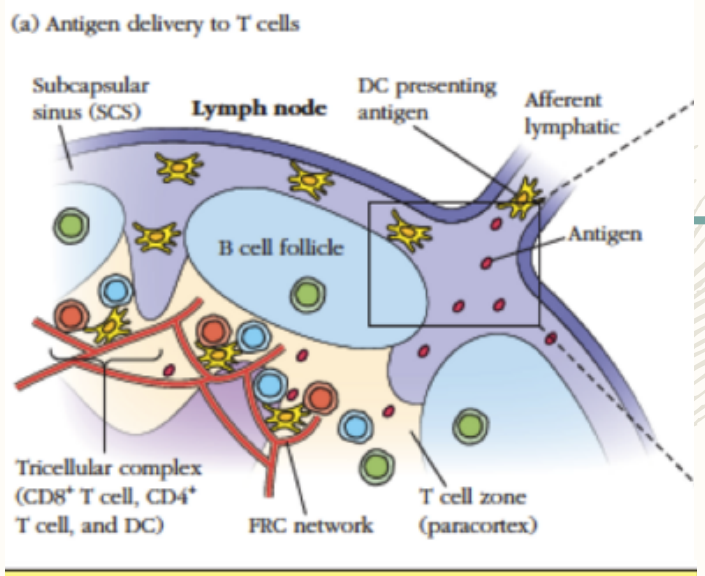
Transition to Adaptive Responses – C3 convertase

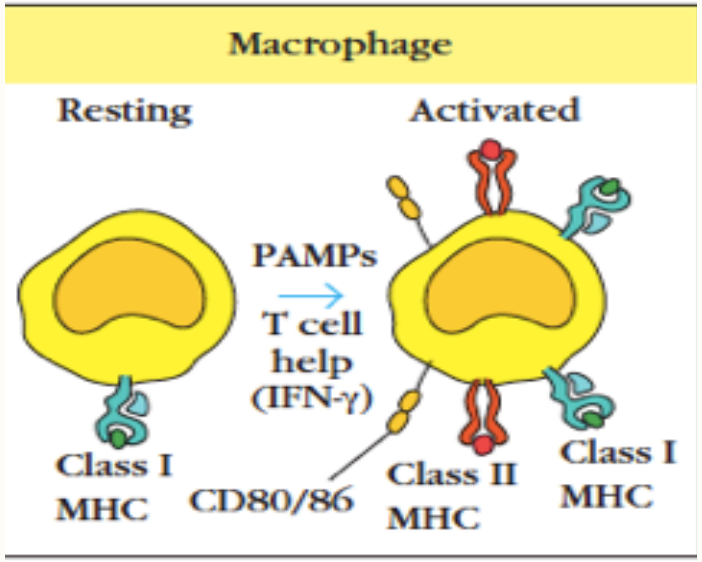
- **C3 convertase**
  - Cleaves C3 into C3a and C3b
  - C3b recruit proteins to form C5 convertase
  - C5 convertase cleaves C5 into C5a and C5b
  - C5b recruit proteins to form Membrane Attack Complex (MAC)

In conclusion, C3 convertase causes

- Inflammatory response
- Opsonization and enhancement of phagocytosis
- Formation of MAC and bacterial cell lysis

3. Adaptive Responses – *M. tuberculosis*





- **Resident DCs** take up and degrade infected apoptotic cells
- DCs increase MHC II expression to present antigenic peptides
- Activated DCs also migrate to local lymph nodes (involving CCL19, CCL21, IL-12p40, IL-12P70)
- DCs present antigenic peptides to **naïve T cell**
- Activated naïve T cells differentiate by co-stimulation of B7/CD28 and IFN-y
- **T helper (Th) 1 cells** produce TNF-B, IL-12, IL-10
- IL-12 stimulate naïve CD4+ T cells to produce IFN-y and its differentiation into Th1 cells
- IFN-y and CD40 ligand provided by Th1 cells activate **macrophages**
- Macrophages produce TNF to activate other macrophages through interactions with TNFR1
- Reactive oxygen species and proteases produced by macrophages cause local tissue damage
- Activated macrophages express higher levels of MHC II, CD40, B7, TNF receptors, and IL-12

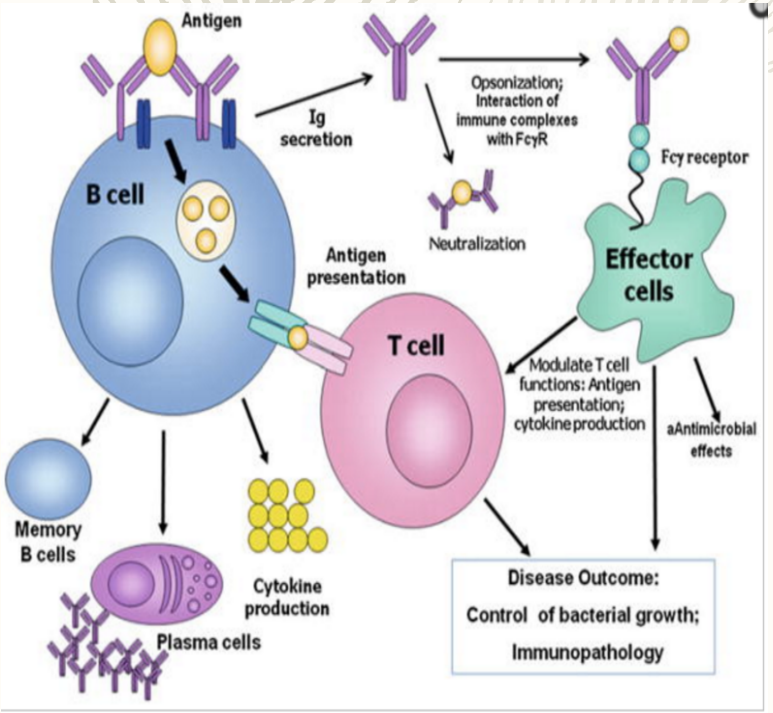
*The humoral immunity against tuberculosis is seen unimportant therefore is not understood

3. Adaptive Responses – *S. pneumoniae*

Cell-mediated Immunity:

- Proteins presented on MHC II of DCs via a nitric oxide dependent pathway are:
  - type I pneumococcal polysaccharide (Sp1)
  - phosphorylcholine (PC) portion of cell wall techoic acid
  - pneumococcal surface protein A (PspA)
- Naïve T cells differentiate into Th2 cells by binding of IL-4 to its heterodimer receptor
- **Th2 cells** express IL-4, IL-5, IL-6, and IL-10
- Th2 cells also help B cell antibody secretion for IgE synthesis/responses
- **Th17 cells** respond to IL-1R1 and IL-23R signaling and are regulated by IL-6, STAT3, RORyt
- Th17 cells produce IL-17A, IL-17F, IL-17AF, IKL-21, IL-22, GM-CSF, MIP-3a, TNF-a that are pro-inflammatory cytokines
- **Follicular B helper cells** can develop with the transcription factors BCL6 and IL-21
- Follicular B helper cells reside in the lymph node to produce mucosal class switched antibodies

3. Adaptive Responses – *S. pneumoniae*



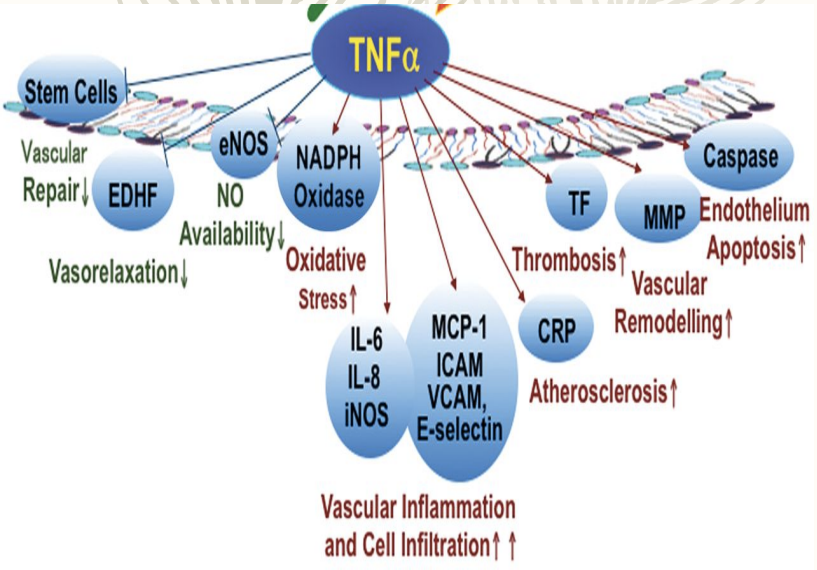
Humoral Immunity:

- Antigen-bound B cell receptor results in an internal signal in the cell
- Th cells provide cytokines and CD40L that induce B cell proliferation and differentiation into antibody (Ab) producing **plasma cells** and **memory B cells**
- **IgA**: a neutralizing antibody that binds to toxins and opsonize bacteria
- Lung epithelial cells trnasport IgA into the airway lumen in the presence of IL-17
- **IgM**: activate complement pathway C3b convertase to opsonize bacteria and infected host cells
- **IgG**: cause inflammation by increasing the cytokine secretion and blood vessel permeability
- The resulting permeable vessel allows myeloid cells to enter the infection site
- IgG is seen at large in alveoli

Question 2 – Host Damage

What damage ensues to the host from the immune response?

M. tuberculosis



TNF-α :

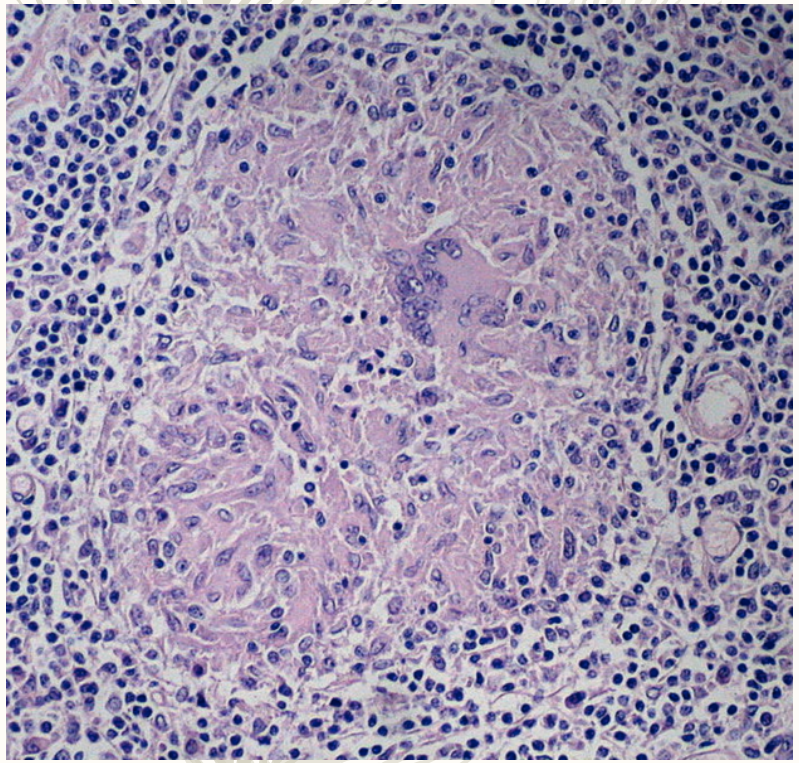
- cytotoxic to epithelial cells
- reduces the production of surfactant protein by type II epithelial cells
- promotes fibroblast activity and enhances the production of fibroblast collagenases
- promotes the production of reactive oxygen intermediaries that are cytotoxic to tissues
- Creates tissue damage (edema and necrosis) of M. tuberculosis lesions leading to organ dysfunction
- activates adhesion molecules on the immunocytes
- Some of the constitutional signs and symptoms of tuberculosis (e,g. night sweats, fever) is indicative of the presence of excess circulating inflammatory cytokines
- High TNF-α and other pro-inflammatory cytokine levels have been reported to correlate with the extent of cavity formation

M. tuberculosis

Excessive production of **TGF-β**:

- associated with extensive fibrosis and tissue damage
- a strong inhibitor of epithelial and endothelial cell growth
- while it promotes the production and deposition of collagen matrix, it also has been shown to increase the production of macrophage collagenases (enzyme that breakdown collagen; contribute to tissue damage)

M. tuberculosis



Granuloma H&E staining

Granulomatus B cell aggregates could contribute to the development of tissue-damaging immunopathology observed in tuberculosis

Granuloma, an aggregate of infected macrophages and neutrophils, forms in the lung to keep the infection localized. However, latent infection causes scarring on the lungs

S. pneumoniae

- If alveolar macrophages fail to clear neutrophils, the following can occur:
  - Neutrophil necrosis
  - the release of reactive oxygen species and proteases
  - subsequent tissue injury like acute lung injury and acute respiratory distress syndrome
- Other pro-inflammatory cytokines such as IL-1, IL-6, and TNF can cause damages
- Pneumolysin and hydrogen peroxide released by *S. pneumoniae* are cytotoxic and induce nitric oxide production, which can in turn lead to septic shock

Question 3 – Bacterial evasion

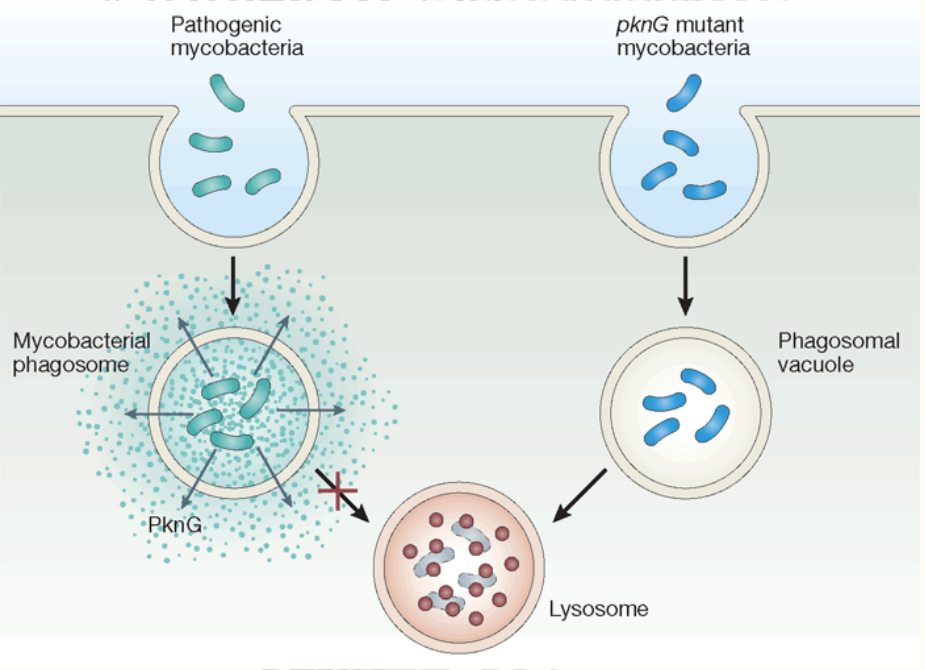
How do the bacteria attempt to evade these host response elements?

M. tuberculosis

As an intracellular pathogen, it avoids host response elements by these methods:

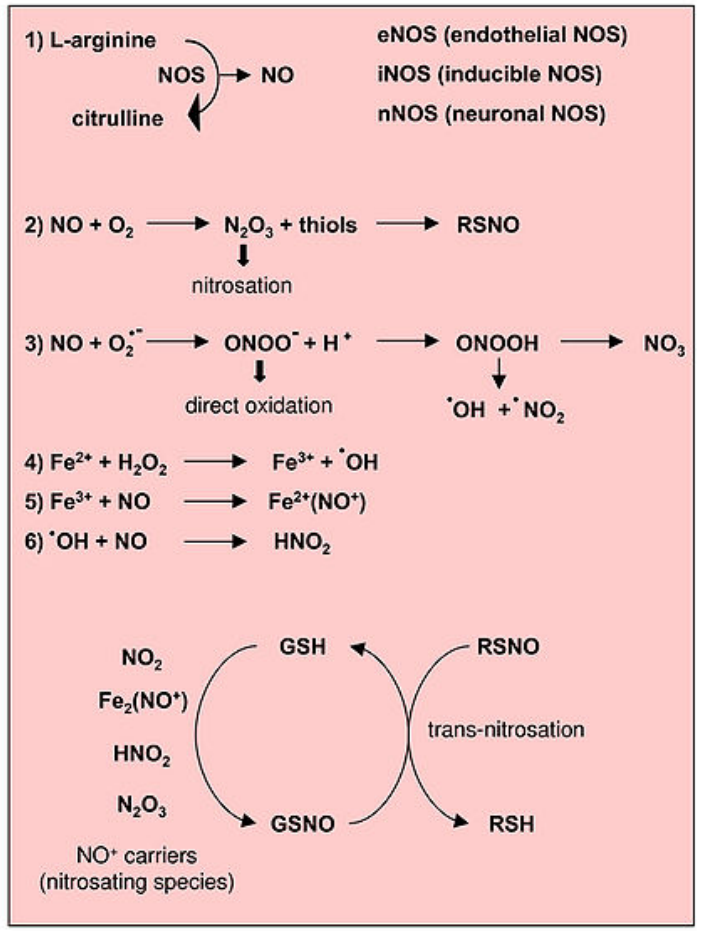
1. Disrupting phagosome-lysosome fusion
2. Resisting reactive oxygen species (ROS) and reactive nitrogen species (RNS)
3. Inhibiting antigen presentation
4. Persisting in a latent form
5. Disrupting Immune Cells

M. Tuberculosis - Disrupting phagosome-lysosome fusion



- *M. Tuberculosis* prevents phagosome-lysosome fusion by inhibiting phagosome maturation.
- Mycobacterial LAM reduces the effect of VP35 by disrupting PI3K signaling
- VP35 normally recruits early endosome antigen 1 (EEA1)
- VP35 and EEA1 normally interact with SNARE, important protein for membrane fusion

M. Tuberculosis – Resisting ROS and RNS



ROS produced by macrophages disrupt bacterial lipids, DNA, proteins, and enzyme functions

The image on the left displays reactions leading to reactive oxygen species (ROS)

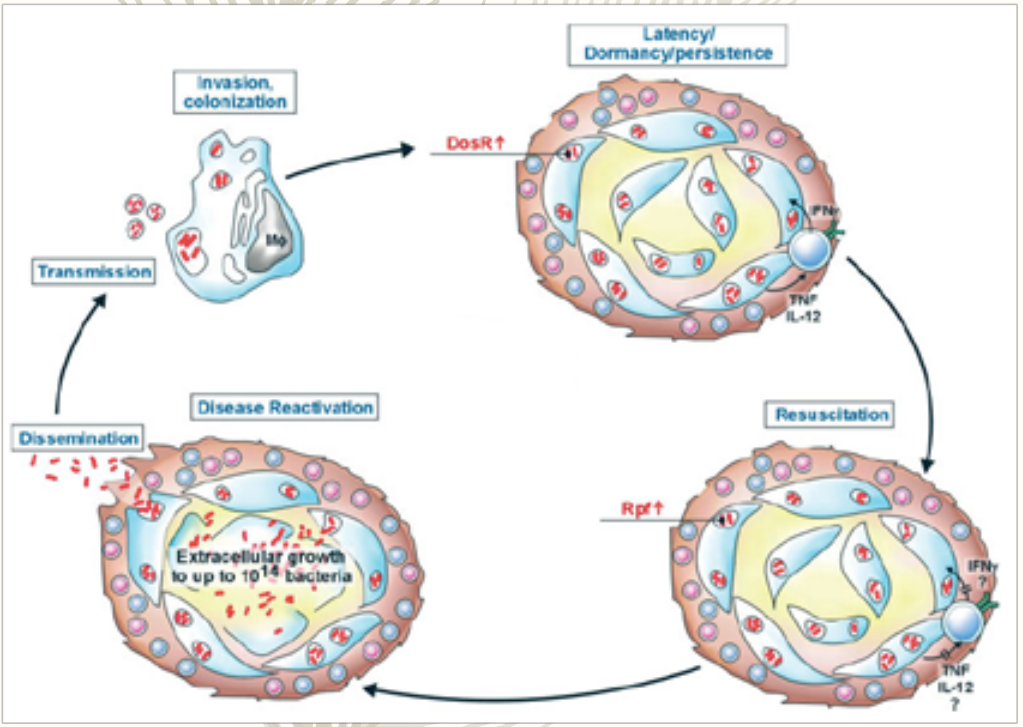
Also, P1-Type ATPases expressed by *M. tuberculosis* provides its resistance to zinc poisoning in macrophages

M. Tuberculosis – Inhibiting antigen presentation

M. tuberculosis inhibit antigen presentation of MHC II for activation of CD4+ T cells

- *M. tuberculosis* downregulates Class II transactivator (CIITA) to inhibit MHC II expression and antigen presentation
- Phagosome maturation arrest to inhibit lysosomal fusion also prevents macrophages from doing antigen presentation
- TLR2 bound by 19kDa lipoprotein on macrophages and phagosomes is thought to downregulate MHC II expression
- 19kDa lipoprotein also induce macrophage apoptosis via TLR-2 dependent pathways
- 19kDa lipoprotein and LAM inhibit IFN-y –induced MHC II upregulation

M. Tuberculosis – Persisting in a latent form

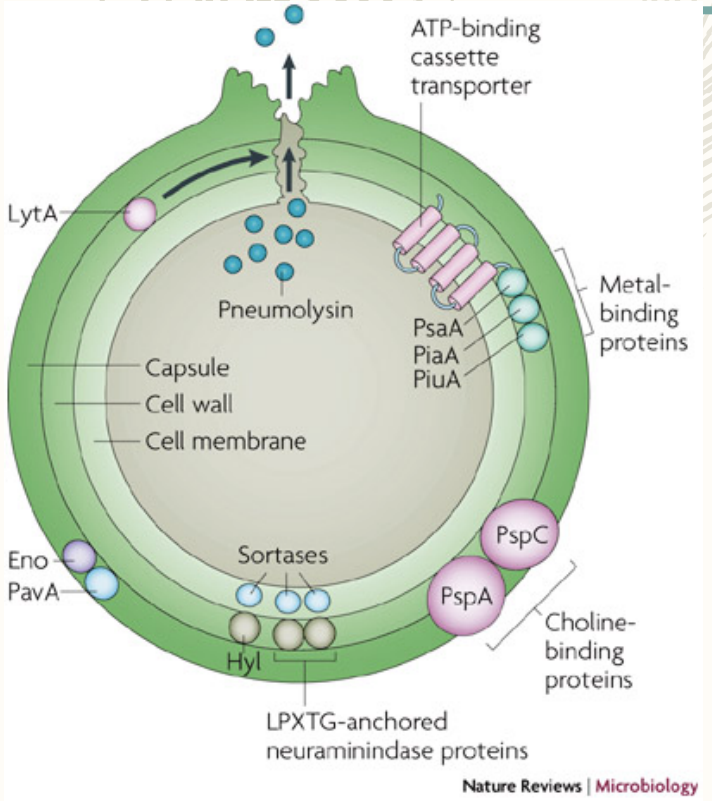


- *M. tuberculosis* can remain latent by increasing cross-linking in its peptidoglycans, reducing cell wall permeability
- Within granulomas, it will remain inactive until the host immune system weakens
- Resuscitation promiting factors (RPF) assist the pathogen to reactivate by hydrolyzing peptidoglycan in conjunction with other proteins.

M. Tuberculosis – Disrupting Immune Cells

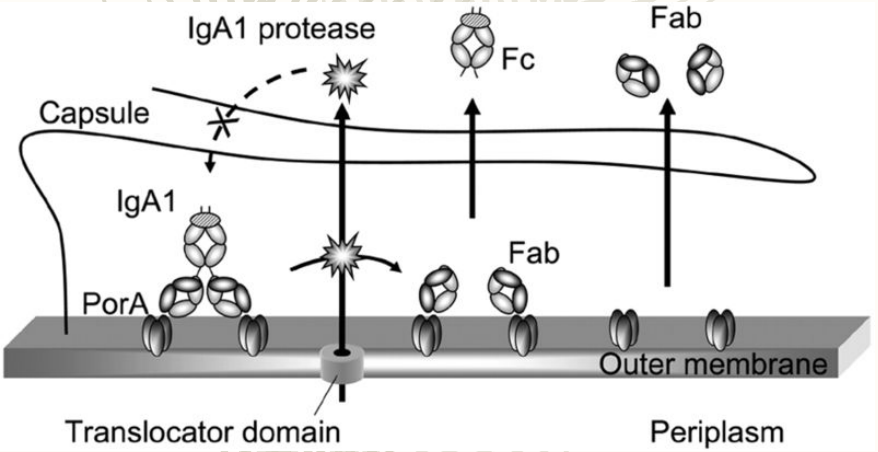
- *M. tuberculosis* induce TGF-B from monocytes and DCs
  - TGF-B inhibit T-cell proliferation and IFN-y production to weaken the inflammatory response
- *M. tuberculosis* upregulate IL-10 secretion from macrophages
  - IL-10 is an anti-inflammatory cytokine that depresses the IFN-y response

S. pneumoniae



- Pneumococcal surface proteins (Psp)
  - PspA: inhibit deposition of C3 due to its electronegative properties and avoid opsonization; also protect against oxidative stress
  - PspC: bind to factor H and disrupt C3b formation in the alternative complement pathway
- Pneumolysin
  - Inhibit ciliary beating of the respiratory epithelium and respiratory burst activity, hydrogen peroxide production and degranulation
- Mutation
  - *S. pneunomiae* undergoes mutation through horizontal genetic exchange and display resistance towards antibiotics

S. pneumoniae



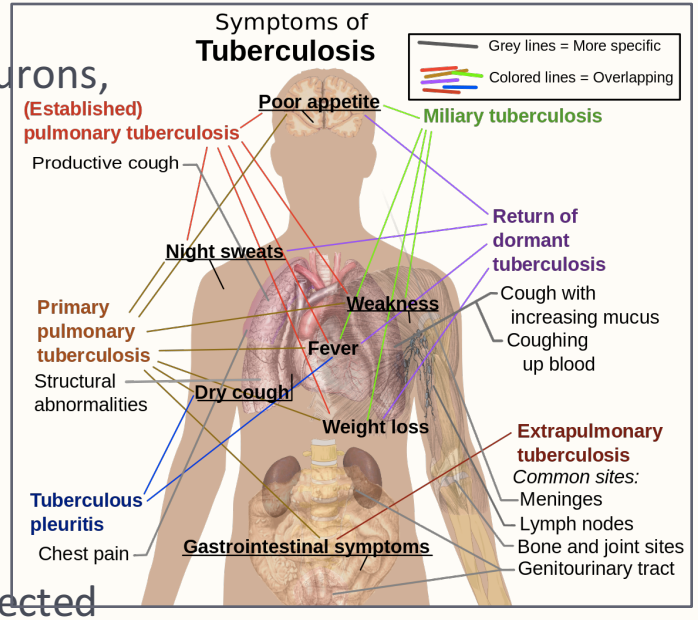
- Capsular polysaccharide
  - Its charged form at a high pH inhibits host's phagocytic function
  - The charge makes the pathogen repulsive to mucopolysaccharides in host mucus
  - Reduction of neutrophil extracellular trap effectiveness
  - Reduced binding to IgG and deposition of C-reactive protein and C3b
  - Pneumoccocal capsule therefore reduce effectiveness of classical and alternative complement pathwy and opsonization.
  - Capsules also restrict autolysis and resist to several antibiotics
- IgA1 Protease (in the left figure)
  - Cleaves human IgA1 bound to PorA to enhance epithelial cell adhesion
  - Cleaved Fab and Fc prevents the host from recognizing the pathogen and subsequently inhibits the inflammatory response

Question 4 - Outcome

Is the bacteria completely removed, does the patient recover fully and is there immunity to future infections with these candidate infectious agents?

M. tuberculsis

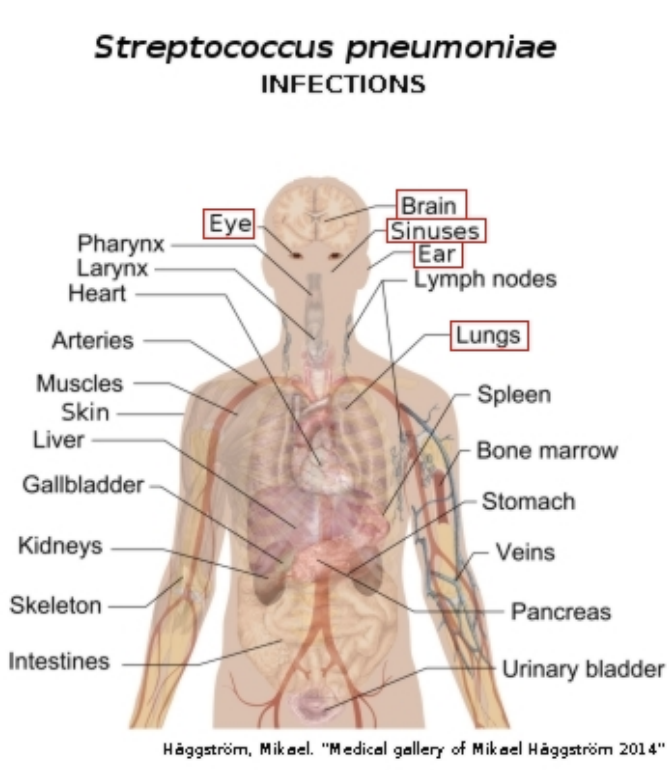
- *M. tuberculosis* can stay inactive for a long time before becoming active, usually as co-infection with other diseases/malnutrition
- *M. tuberculosis* can bacteremically spread to secondary sites via circulation, affecting neurons, adrenal glands, cardiac and gastrointestinal tissues.
- Secondary lesions can occur in the lungs when *M. tuberculosis* re-inoculate
- Antibiotics are used to treat tuberculosis
- Due to its ability to stay dormant, reoccurrence rates are high
- Overuse of drugs can lead to multi-drug resistant strains over time
- Individuals may develop life-time risk for re-infection
- Preventative measures include practicing proper hygiene, ventilating rooms, avoiding infected individuals, etc.
- BCG (Bacillus Calmette-Guerin) vaccine is given to children to elicit an adaptive immune response against this bacterial infection



M. Tuberculosis - antibiotics

Drugs	Description	Usage
Isoniazid	Prevents bacterial fatty acid synthesis by the production of reactive nitric oxides	For an active infection: 5mg/kg IM or orally daily For latent infection: 10-20mg/kg daily *do not exceed 300mg/day
Pyrazinamide	Its active form, pyrazinoic acid binds to ribosomal protein S1 (RpsA) and inhibit trans-translation	Used with other antibiotics to shorten the duration of treatment
Rifampicin/priftin	Inhibits bacterial DNA-dependent RNA polymerase (mRNA transcription)	600mg twice a week for 2 months 600mg/week for 4 months
Ethambutol	Disrupts bacterial cell wall synthesis, which compromises bacterial membrane integrity	Taken first three months

S. pneumoniae



- *S. pneumoniae* undergoes antigenic variation frequently
- It can remain undetected and spread to secondary sites
- The generation of memory T and B cells cannot completely remove *S. pneumoniae* infection in the future
- Antibiotic treatment is necessary to combat *S. pneumoniae* infection
- The broad-spectrum cephalosporin is recommended to be used until antibiotic sensitivity is determined (CDC)
- However, bacteria develop antibiotic resistance due to their extremely high replication rate and large cell densities that may cause mutations

S. Pneumoniae - antibiotics

Drugs	Description
Macrolides (azithromycin)	Inhibits the growth of bacteria by binding to the 50S subunit during mRNA translation; azithromycin and amoxicillin are the two most common and effective oral antibiotics of s. pneumonia infection with a cure rate of 80%
Penicillin (amoxicillin)	Inhibits cell wall biosynthesis, disrupting membrane integrity
Fluroquinolones	Inhibits bacterial DNA from unwinding and duplication, preventing DNA replication
Tetracyclines	Bind to ribosomal 30S subunit, inhibiting protein translation
Vancomycin	Glycopeptide that inhibits proper cell wall synthesis

S. pneumoniae

- For patients with any co-morbidities (immunosuppressing conditions or chronic heart disease), respiratory fluoroquinolones and B-lactams are recommended
  - Some resistance to these drugs have emerged, however
- Individuals may be faced with re-infection if memory T and B cells are unable to recognize altered antigen of the new serotype due to common antigenic variations
- Preventative measures include practicing proper hygiene and avoiding smoking
- Pneumonococcal polysaccharide vaccine (PPSV23) with 23 different bacterial strains of bacteria is offered, however, these vaccines do not elicit a very strong antibody response as immune response only occurs against the capsular polysaccharides and not proteins
  - Polysaccarides are less immunogenic than proteins