PATH 417

Case 3: From India to Canada

Immune Response Questions

Lindsay Richter

The Case:

The 53-year-old patient reports experiencing fevers, chills, night sweats, and a chronic productive cough over the past month. He immigrated from India one year ago.

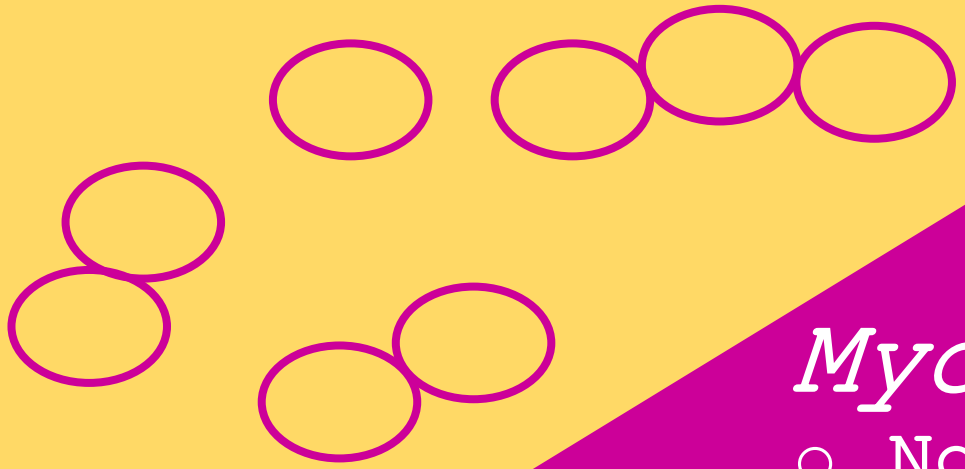
The doctor confirms a fever of 38.5°C, finds crackles in the right lung and decreased breath sounds in the right lower lung field.

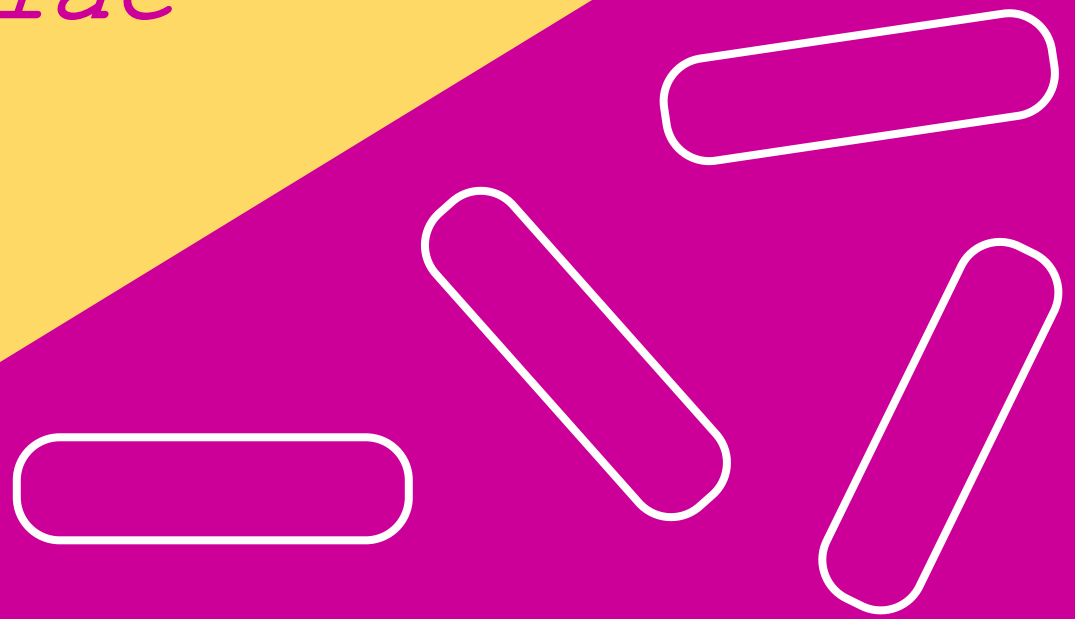
After a chest X-ray and collection of deep sputum samples, the Public Health Unit notifies the patient to report to the local hospital for further assessment.

The likely pathogens:

Streptococcus pneumoniae

- Gram-positive
- Non-sporulating
- Non-motile
- Cocci(pairs or short chains)





Mycobacterium tuberculosis

- Non-sporulating
- Non-motile
- Rod-shaped aerobes

Host Response

Three lines of defense:

1. Anatomical + Physical Barriers
2. Innate Responses
3. Adaptive Responses

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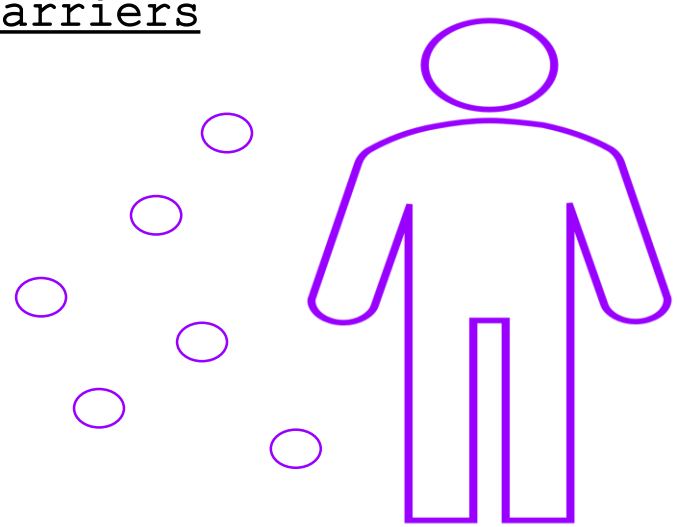
Anatomical + Physiological Barriers

- First line of defense
- Includes:
  - Intact skin
  - Mucous membranes
  - Low pH of stomach
  - Body temperature that inhibits many pathogen's growth

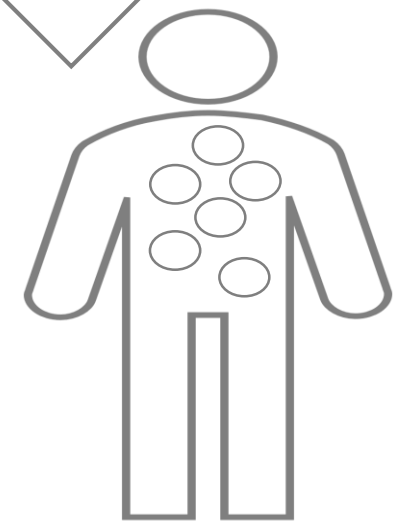


Mucocillary Transport System
- pathogens trapped in mucus
- mucus produced by cilia + goblet cells
- mucosal production increased during infection

-> prevent pathogens from entering lower respiratory tract

→ Also involved in innate immunity

If anatomical + physiological barriers have been breached...



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Innate Immune System

Host Response

2

Innate Immune Response

- Nonspecific
- Main functions:
  - Phagocytosis
  - Opsonisation
  - Activation of complement cascade
  - Chemotaxis of phagocytic cells
  - Activation of inflammatory response

- **Mucus**
  - Lysozyme -> degrade glyosidic bonds of peptidoglycan
  - Secretory IgA -> bind, neutralize, trap pathogens in mucus
  - Lactoferrin -> sequesters iron from pathogens
  - Lactoperoxidase -> generates toxic superoxide radicals
- **Cilia**
  - Rhythmic waving motion to move mucus up and out of airways -> then swallowed + pathogens are killed by acidic stomach pH
- **Antimicrobial proteins**
  - Surface proteins SP-A + SP-B -> block bacterial surface components + promote phagocytosis
  - Defensins Alpha + Beta -> hydrophobic regions allow them to pass through bacterial membranes, aggregate in dimers, form a pore complex to cause membrane depolarization + lysis
  - Cathelicidin LL-37 -> inserts in membranes to cause lysis
- **Neutrophils**
  - Recruited from bone marrow into blood stream by cytokines
  - Move via diapedesis, kill via phagocytosis, degranulation + neutrophil extracellular traps

- **Pathogen Recognition Receptors**
  - PRRs -> divided into phagocytosis receptors or Toll-like receptors (induce cell activation + cytokine production)
  - PAMPs -> include LPS, mannose, bacterial RNA/DNA, peptidoglycans, lipoteichoic acids, lipoproteins, flagellin, microtubules
  - In this case, important TLRs include:
    - TLR2 -> binds peptidoglycan + lipoproteins
    - TLR4 -> binds pneumolysin (*S. pneumoniae* secreted toxin)
    - C-type lectin receptor -> bind mycobacterial glycolipid trehalose dimycolate (TDM) to produce alarm cytokines (IL-6, TNF-a, IL-1)
  - NOD2 -> sense muramyl dipeptide (MDP)
- **Lysosomes**
  - Endosome fuse with lysosome
  - Degraded by reactive oxygen or nitrogen species
  - Pathway carried out by macrophages, neutrophils + dendritic cells

Signalling Pathways

- When TLRs binds -> activates NF-kB -> production of Type interferons (alpha + beta) -> activates NK cells, macrophages, T cells -> secrete cytokines
- TNF-a -> activates macrophages + increases vascular permeability
- TNF -> transmembrane protein that binds TNFR1 + TNFR2 -> induce anti-apoptotic, pro-inflammatory signals by activating mitogen activated protein kinase/ nuclear factor
- TNR1 -> induce apoptotic + anti-inflammatory via caspase 8 + Fas associated death domain.

Host Response

3 Adaptive Immune Response

- Initiated by dendritic cells -> DCs interact with PAMPs + FC receptors which stimulates maturation of DC -> increase MHC + granule antigens on surface, cytokine production + expression of CD80/CD86=B7
- MHC II present the antigenic peptides to TCR -> leads to transcription of IL-2 + binding of B7 to CD28 on T-cell
- Activates naïve Tcells to mature cytotoxic or helper Tcells

- The Complement System

- Composed of serum proteins that act together in catalysis cascades to kill extracellular pathogens
- Include: opsins, membrane attack proteins, complement receptor proteins + initiatory molecules
- Activated via one of three pathways:

1. The Classical Pathway

- When IgM or IgG antibodies from past adaptive immune response bind to pathogens surface -> forms C3 convertase

2. The Lectin Pathway

- Mannose binding lectin (MBL) and ficolins (Abs) recognize PAMPs -> generates C3 convertase -> cleaves C3 into C3a, C3b, C3b, C5a, C5b

3. The Alternative Pathway

- C3 is spontaneously self-hydrolyzed, changes shape, binds Factor B -> cleaved to Bb and Ba by Factor D
- Alternative pathway C3-convertase = Bb + C3(H20)

C3 convertase -> cleaves C3 to C3a + C3b -> C3b binds surface + recruits to form C5 convertase -> cleaves C5 to C5a + C5b -> binds to surface with C2b to initiate the assembly of late complement proteins to form the MAC

1. Inflammatory Response

- C3a + C5a -> cause resident macrophages + mast cells to release histamine + TNF-a
- C5a -> chemoattractant for leukocytes
- Redness, heat, swelling, pain

2. Opsonisation

- C3b as an opsonincauses alteration of surface to be engulfed easily

3. Membrane Attack Complex

- Assembly of MAC with C5b on surface -> punctures surface + causes lysis

Products of C3 convertase actions

S. pneumoniae

- Cell wall features that elicit adaptive response: Sp1, PC + PspA
- Anti-PC AB response occurs earlier than anti-PspA response + is dependent on non-cognate activation of TCR-nonspecific Tcells
- Th2 cells helps with Bcell AB secretion for IgE synthesis/responses
- Th17 cells -> produce IL17A, IL17F, IL22, GMCSF, MIP3a, TNFa -> required in lungs for mucosal immunity
- Follicular B helper cells critical in production of mucosal class switched Abs
- Humoral immunity is vital
- Lung epithelial cells express polymeric Ig receptors, transport IgA into airway lumen in presence of IL17
- IgM activate complement
- IgG increases inflammatory response -> allows myeloid cells to enter infection site -> high levels of IgG in alveoli

M. tuberculosis

-infected cells taken up by DCs -> activates Tcells -> produce TNFB, IL12, IL10

-antigen specific Th1, CD1 restricted Tcells + NK cells produce IFNy -> macrophages secrete TNF + produce ROS and proteases ->localized tissue damage

-IL12 causes more IFNy to be prpduced by Th1 cells

-humoral response considered unimportant + role remains not well understood

Host Damage

M. tuberculosis

Damage caused by TNF-α
- Cytotoxic to epithelial cells
- Reduces production of surfactant protein by type II epithelial cells
- Promotes fibroblast activity + production of fibroblast collagenases
- Promotes production of reactive oxygen intermediates -> cytotoxic -> enhances damage
- Involved in the tissue damage (edema and necrosis) of lesions -> organ dysfunction
- Excess circulating inflammatory cytokines -> night sweats, fever etc.

Damage caused by TGF-β
- Leads to extensive fibrosis + tissue damage
- Strong inhibitor of epithelial + endothelial cell growth -> increase production of macrophage collagenases -> breakdown collagen -> tissue damage

Damage caused by B cells
- Perpetuation of the local immune responses by granulomatous B cell aggregates -> development of tissue-damaging immunopathology observed in tuberculosis

S. pneumoniae

Failure of alveolar macrophages to clear neutrophils
- Results in neutrophil necrosis, release of reactive oxygen species + proteases, and subsequent tissue injury + inflammation

Other tissue damage caused by pro-inflammatory cytokines produced due to inflammation reactions -> similar to ones discussed in *M. tuberculosis*

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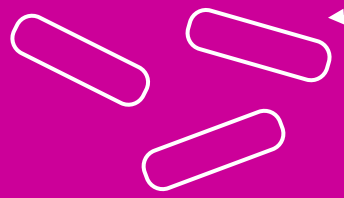
Bacterial Evasion

M. tuberculosis



Intracellular pathogen

Avoids exposure to Abs + complement by replicating within macrophages

Can resist + evade the innate + adaptive immune system by:

(A) Disrupting phagosome-lysosome fusion

- Mycobacterial LAM -> reduce effect of VPS34 (kinase recruits EEA1)
- [EEA1 + VPS34: phagosome maturation through interactions with SNARE]

Overview of phagosome-lysosome fusion:

1. Phagosome containing Mtb acquires a GTPase (Rab5)
2. Rab5 recruits the protein VPS34
3. VPS34 generates PI(3)P on cytosolic face of phagosome
4. PI(3)P complexes with early autosomal antigen-1 (EEA1) + Rab5
5. EEA1 recruits GTPase Rab7 (facilitates late fusion with endosomes
6. SNAREs found on transport + destination vesicles mediate vesicle fusion

LAM inhibits VPS34 + disrupts PI(3)P-kinase signalling + EEA1 recruitment

(B) Resisting reactive oxygen + nitrogen intermediates

- AhpC: reductase to catabolize peroxynitrite
- MsrA: reductase to convert methionine sulfoxide into methionine
- NoxR1 + NoxR3: unknown mechanism
- Superoxide dismutase A+C: dismutase that can degrade superoxides
- P1-Type ATPases: resist zinc poisoning

(C) Inhibiting antigen presentation

- Interferes with MHCII antigen presentation by inhibiting gene expression of MHCII + loading of antigen peptide
- Downregulates Class II transactivator
- 19kDa lipoprotein binding to TLR2 thought to downregulate MHCII expression
- LAM may have effect of inhibiting IFNy-induced MHCII

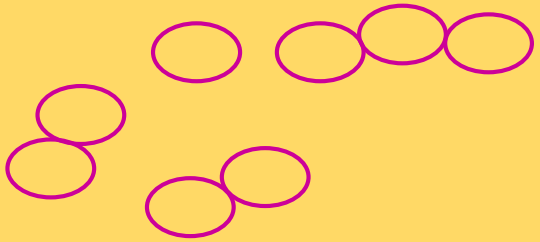
(D) Persisting in a latent form

- Increase cross-linking in PG -> reduce permeability of cell wall + increase survival in environmental duress
- Remain inactive in scarred granulomatous lesions
- Rpf: reactivate by hydrolyzing PG with another unknown protein

(E) Disrupting immune cells

- Induce TGF-B from monocytes + DCs -> inhibit Tcell proliferation + IFN-y production -> weaken inflammatory response
- Upregulate IL10 production in macrophages -> anti-inflammatory cytokine -> decreases IFN-y response

Bacterial Evasion



S. pneumoniae

Capsular Polysaccharide
- Charged at high pH -> anti-phagocytic + disruptive to phagocyte function
- Increases repulsion to mucopolysaccharides in mucus
- Reduces neutrophil extracellular trap effectiveness
- Reduces binding to Fc region of IgG + inhibits conversion of bound C3b -> reduces effectiveness of classical + alternative complement pathway
- Functions to restrict autolysis + resist antibiotics

Pneumolysin
- Inhibits ciliary beating of respiratory epithelium -> limits ability to be expelled from lung
- Inhibits respiratory burst activity, hydrogen peroxide production + degranulation

Pneumococcal Surface Proteins
- PspA -> inhibit deposition of C3 due to its electronegative properties -> avoid opsonisation -> also protect against oxidative stress
- PspC -> a H-binding inhibitor of complement -> bind to factor H -> disrupt C3b formation in alternative complement pathway

IgA1 Protease
- Zinc metalloproteases: cleaves IgA1 which increases the changes of epithelial cell adhesion -> cleaves fragment antigen binding (Fab) -> bound to pneumococcal antigen from Fc portions of AB -> prevents host from recognizing/ initiating inflammatory response

Mutation
- Mutation + quick growth aids in antibiotic resistance
- Also capable of horizontal genetic exchange

Outcome

M. tuberculosis

- Can lay dormant before becoming active with environment changes
- Untreated -> spread from blood cause secondary infections (affecting neurons, adrenal glands, cardiac and gastrointestinal tissues + re-inoculate the lungs to cause secondary lesions

- Aggressive antibiotic treatment: isoniazid, pyrazinamide, rifampicin/priftin + ethambutol (3 months) -> isoniazid + rifampicin/priftin (6-12 months)
- Pyrainamide: binds RpsA + inhibit translation
- Ethambutanol: compromise membrane integrity
- Isoniazid: prevents fatty acid synthesis

- Reoccurrence rates high -> persistence state
- Complete immunity only achieve for short time
- Rise in multi-drug resistant strains w/overuse
- Individuals diagnosed -> lifetime risk for re-infection -> highest in first 5 years
- Preventative control measures needed: proper ventilation, proper hygiene, wearing a mask + advising infected individuals to stay home
- Vaccination of young children with Bacillus Calmatte-Guerin -> elicit adaptive immune response to Mtb infection/preventing progression

S. pneumoniae

- High frequency to undergo antigenic variation + spread to cause secondary infections
- Untreated -> can evade host immune responses for the duration of host's life
- Antibiotic treatment is necessary: macrolides, tetracyclines, fluoroquinolones, penicillin + vancomycins
- Macrolides: inhibit growth by binding 50S
- Penicillin: disrupt membrane integritiy
- Fluroquinolones: prevent DNA replication
- Tetracyclines: inhibit protein translation
- Vancomycin: inhibits cell wall synthesis
- Antibiotic resistance due to its extremely high replication rate + large cell densities
- Not possible to be immune for future infections -> 90 serotypes + capsule switching to change antigenic expression
- Preventative measures: hand hygiene + avoiding smoking
- Vaccine: PSSV23 - 23 strains -> elicits immune response against capsular polysaccharides -> cross-reactivity possible -> weak antibody response -> polysaccharides are less immunogenic than proteins
- Recently introduced conjugate vaccine