A study material for M.Sc. Biochemistry (Semester: IV) Students
on the topic (EC-1; Unit IV)

Tuberculosis

A Disease of *Mycobacterium*

Vyomesh Vibhaw
Assistant Professor (Part Time)
Department of Biochemistry
Patna University
Mob. No.:- +91-9708381107, +91-8825217209
E. Mail: vyomesh.vibhaw@gmail.com

Tuberculosis

- **Tuberculosis**, **MTB**, or **TB** (short for *tubercle bacillus*) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*.

- Tuberculosis may infect 1/3 of the world population asymptomatically and causes ~ 2 million deaths/year.

- Tuberculosis typically attacks the lungs, but can also affect other parts of the body.

- It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit their saliva through the air.

- Airborne transmission most common, transmission also possible via unpasteurized milk products.

- When people with active pulmonary TB cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5.0 μm in diameter.

- A single sneeze can release up to 40,000 droplets.

- Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very low (the inhalation of fewer than 10 bacteria may cause an infection).

- Most infections are asymptomatic and latent, but about one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of infected persons.

- The classic symptoms of active TB infection are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss.

- Diagnosis of latent TB relies on the tuberculin skin test (TST) and/or blood tests.

- Antibiotic resistance is a growing problem in multiple drug-resistant tuberculosis (MDR-TB) infections.

- Bacille Calmette-Guerin (BCG) vaccine available but variable efficacy for adults.

- Prevention relies on screening programs and vaccination with the bacillus Calmette-Guerin (BCG) vaccine.

- One third of the world population is thought to have been infected with *M. tuberculosis*, with new infections occurring at a rate of about one per second.

- About 5–10% of those without HIV, infected with tuberculosis, develop active disease during their lifetimes.

- In contrast, 30% of those co-infected with HIV develop active disease.

Mycobacterium tuberculosis

- Gram positive, Rod shaped bacilli,
- Reservoir: humans.
- Acid-fast rod; transmitted from human to human.
- *M. bovis*: <1% U.S. cases; not transmitted from human to human.
- *M. avium-intracellulare* complex infects people with late-stage HIV infection.
- Enters host macrophages and subverts normal phagosome maturation.
- Persists in a granuloma

Mycobacteria

Pathogenic species:

M. tuberculosis – obligate intracellular pathogen; infections in humans > animals.

M. bovis – infections in animals > humans.

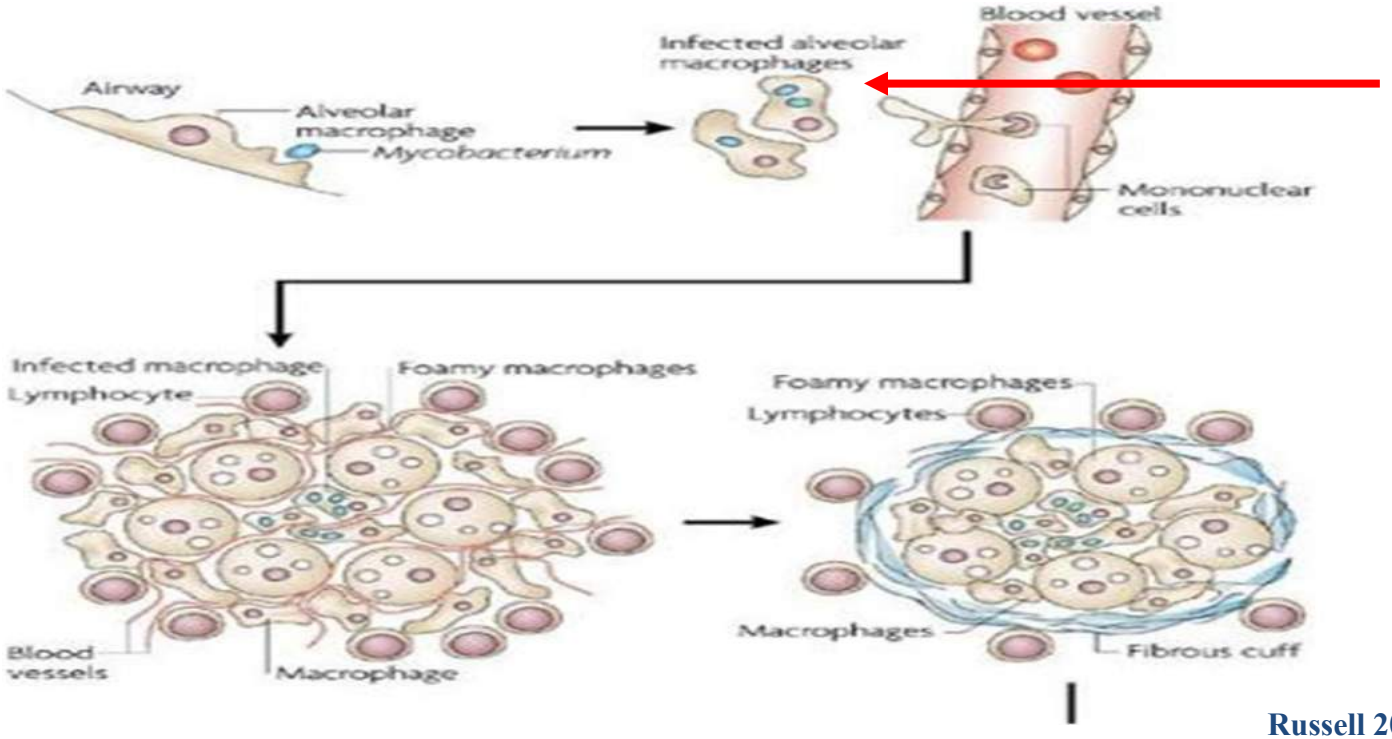
Non-pathogenic species:

M. smegmatis, M. vaccae.

Survive in the environment.

Infected macrophages and the granuloma

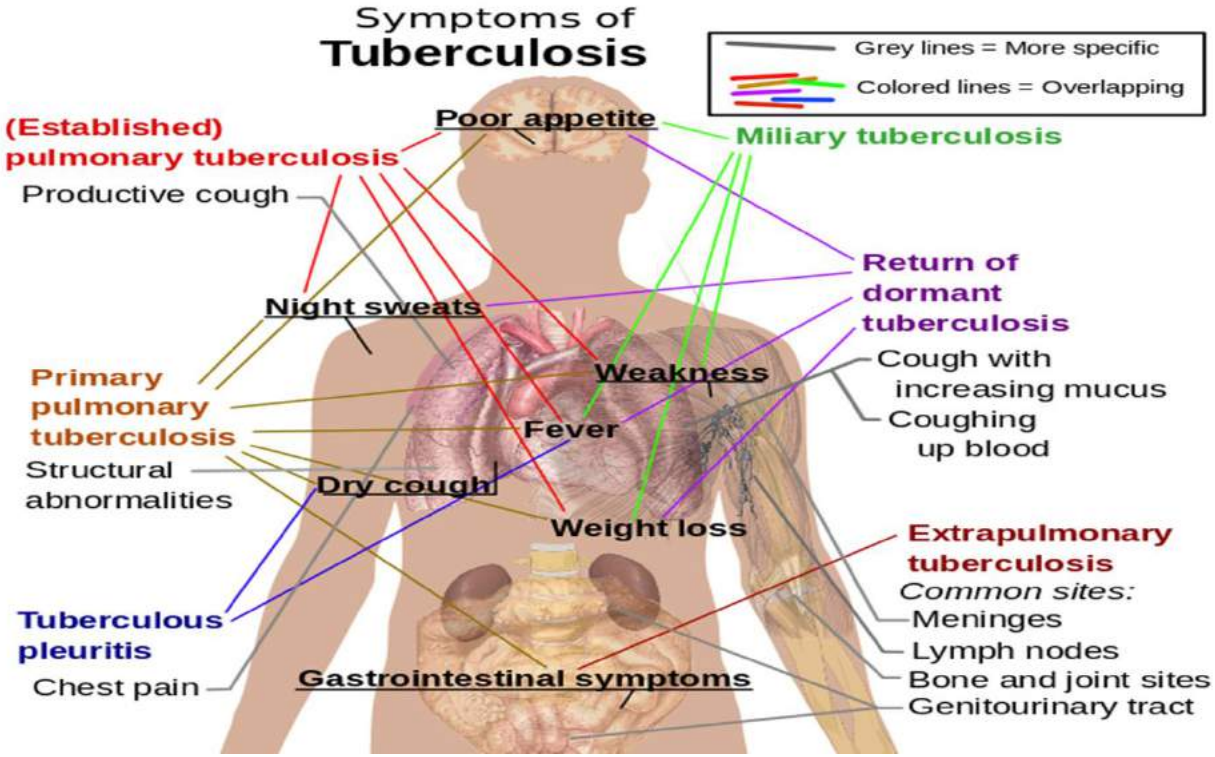
- Persists in a granuloma
- **Granuloma** is a tiny collection of immune cells known as macrophages.
- A granuloma is a small area of inflammation in tissue.
- Granulomas are most often the result of an infection and most frequently occur in the lungs, but can occur in other parts of the body as well.

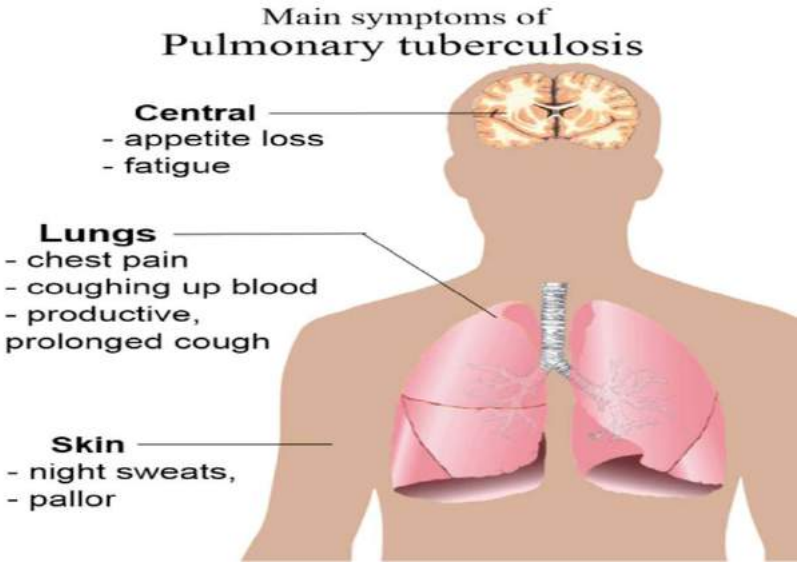


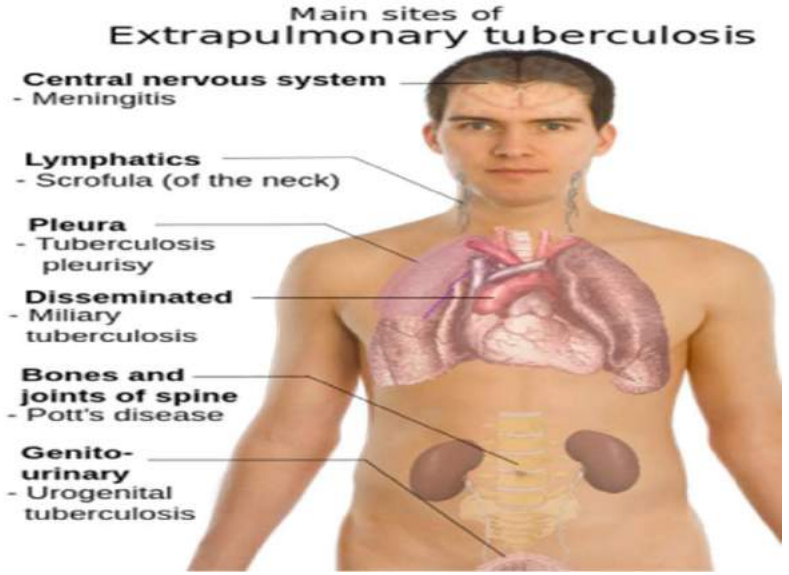
Intracellular
M. tuberculosis

Granuloma

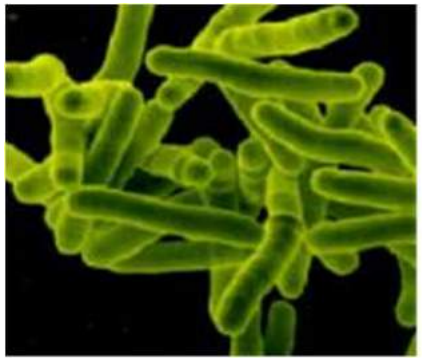
Russell 2007 Nat Rev Microbiol 5:39-47

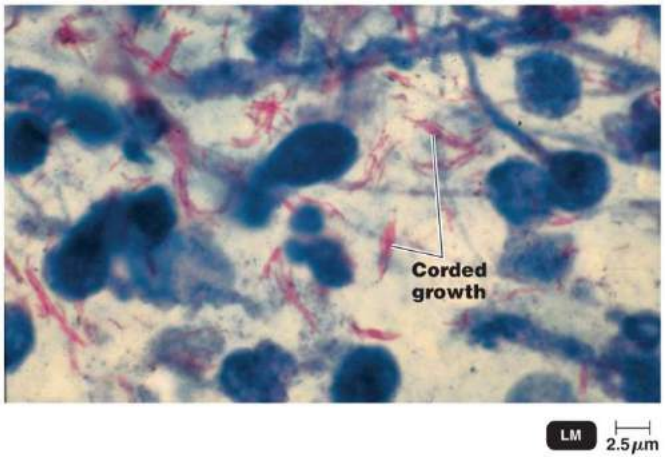

Symptoms of
Tuberculosis
Grey lines = More specific
Colored lines = Overlapping
(Established) pulmonary tuberculosis
Productive cough
Poor appetite
Miliary tuberculosis
Return of dormant tuberculosis
Cough with increasing mucus
Coughing up blood
Night sweats
Primary pulmonary tuberculosis
Structural abnormalities
Weakness
Fever
Dry cough
Weight loss
Extrapulmonary tuberculosis
Common sites:
Meninges
Lymph nodes
Bone and joint sites
Genitourinary tract
Tuberculous pleuritis
Chest pain
Gastrointestinal symptoms


Main symptoms of
Pulmonary tuberculosis
Central
- appetite loss
- fatigue
Lungs
- chest pain
- coughing up blood
- productive, prolonged cough
Skin
- night sweats,
- pallor

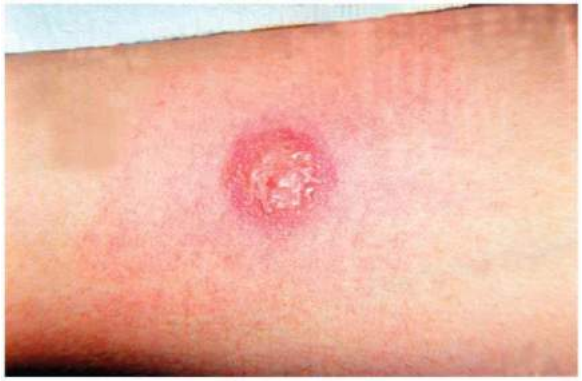

Main sites of
Extrapulmonary tuberculosis
Central nervous system
- Meningitis
Lymphatics
- Scrofula (of the neck)
Pleura
- Tuberculosis pleurisy
Disseminated
- Miliary tuberculosis
Bones and joints of spine
- Pott's disease
Genito-urinary
- Urogenital tuberculosis

Tuberculosis





Positive Tuberculin Skin Test

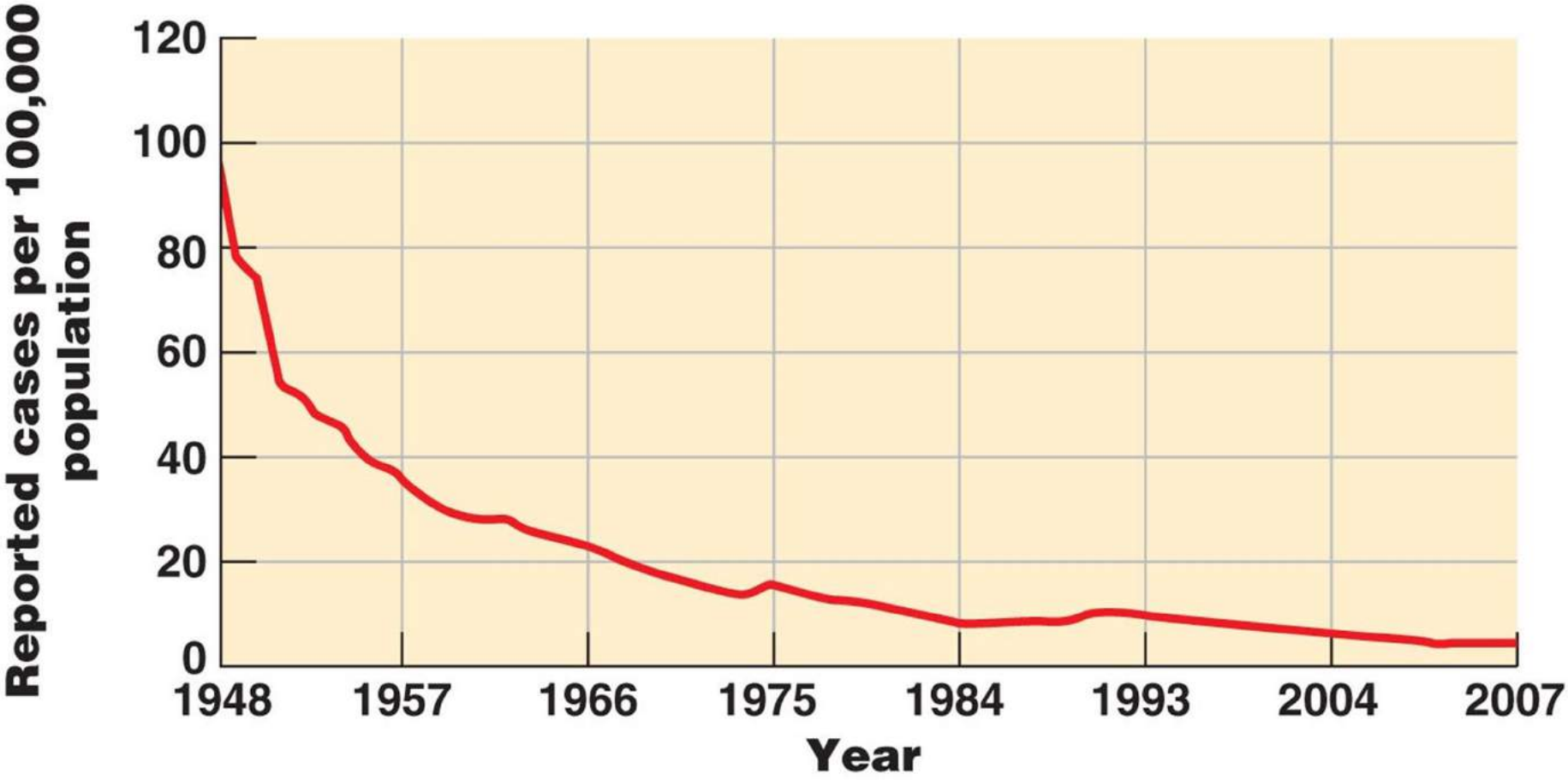


Tuberculin skin test screening

Positive reaction means current or previous infection

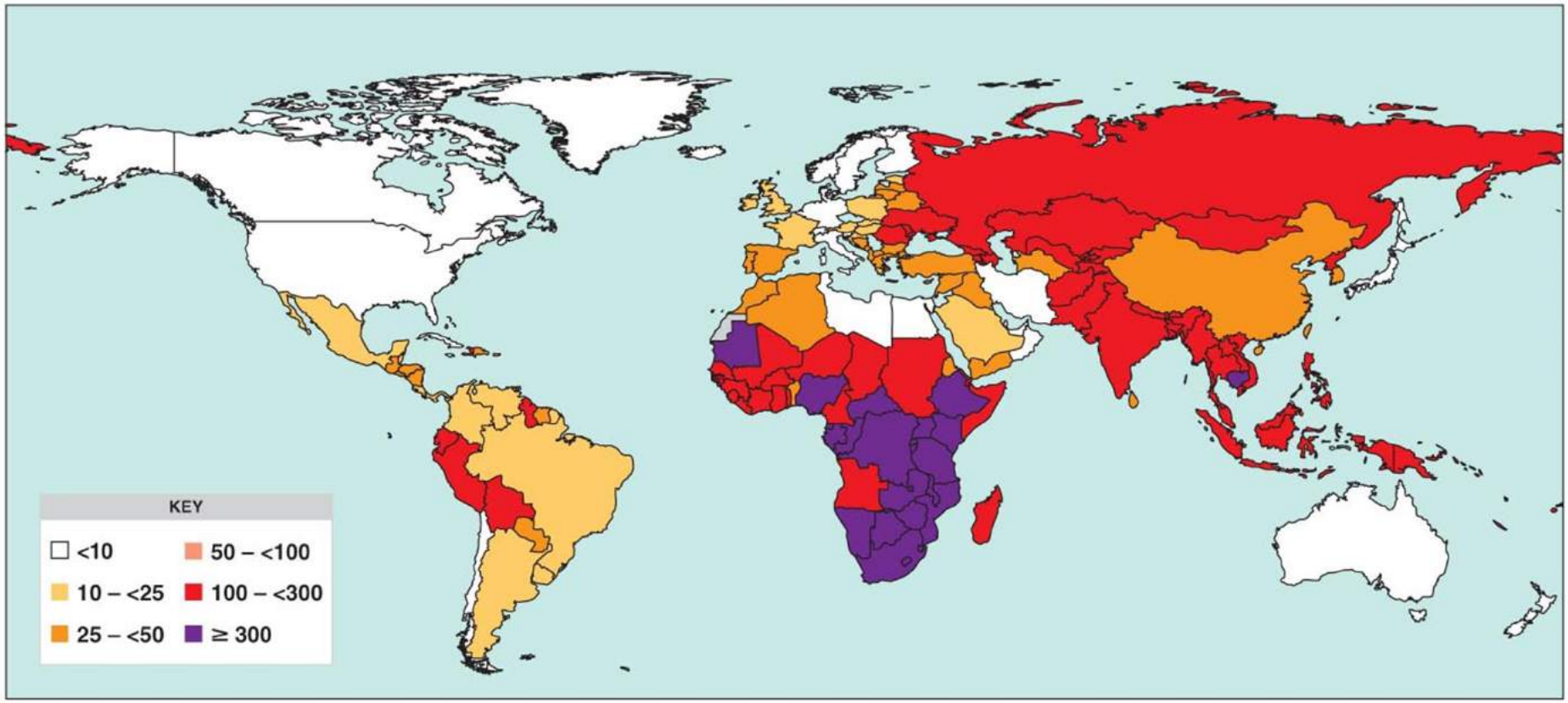
Followed by X-ray or CT exam, acid-fast staining of sputum, culturing of bacteria

Tuberculosis



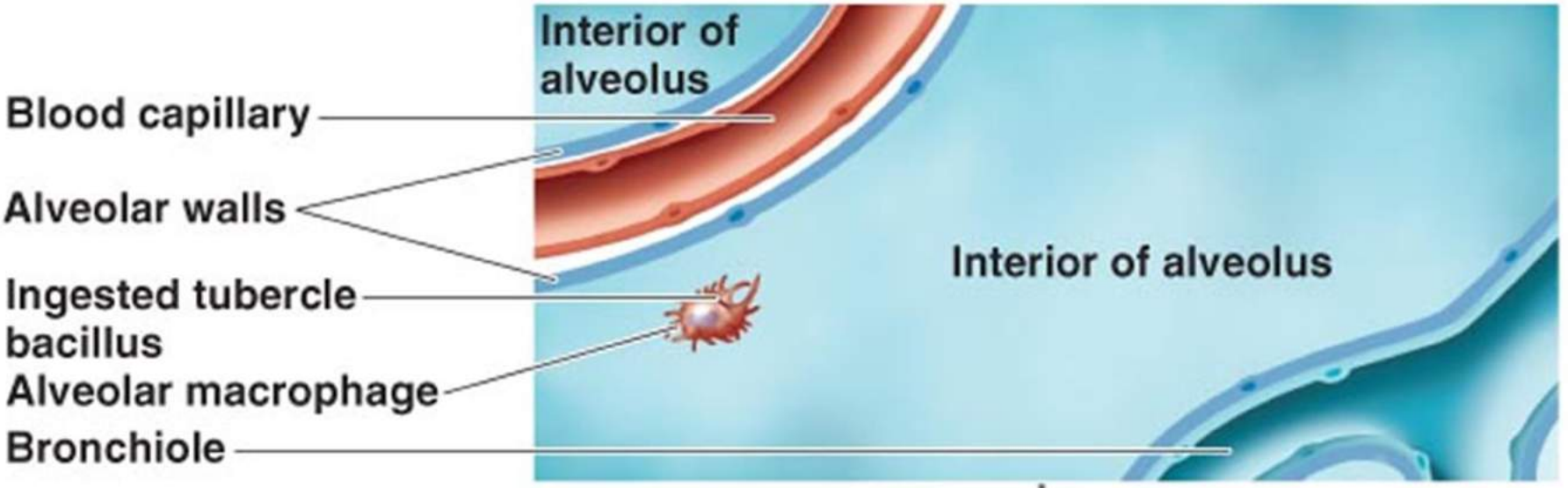
(c) Reported tuberculosis cases, 1948–2007

Worldwide Distribution of Tuberculosis



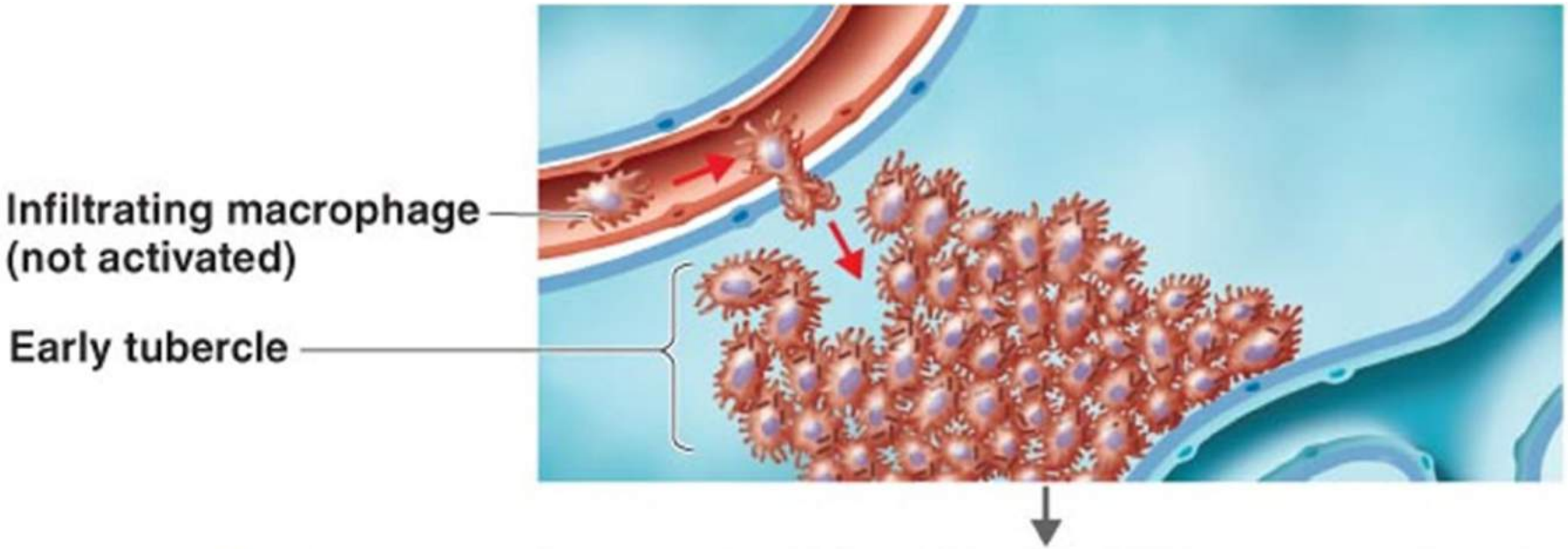
(a) Estimated tuberculosis incidence worldwide, per 100,000 population.

The Pathogenesis of Tuberculosis



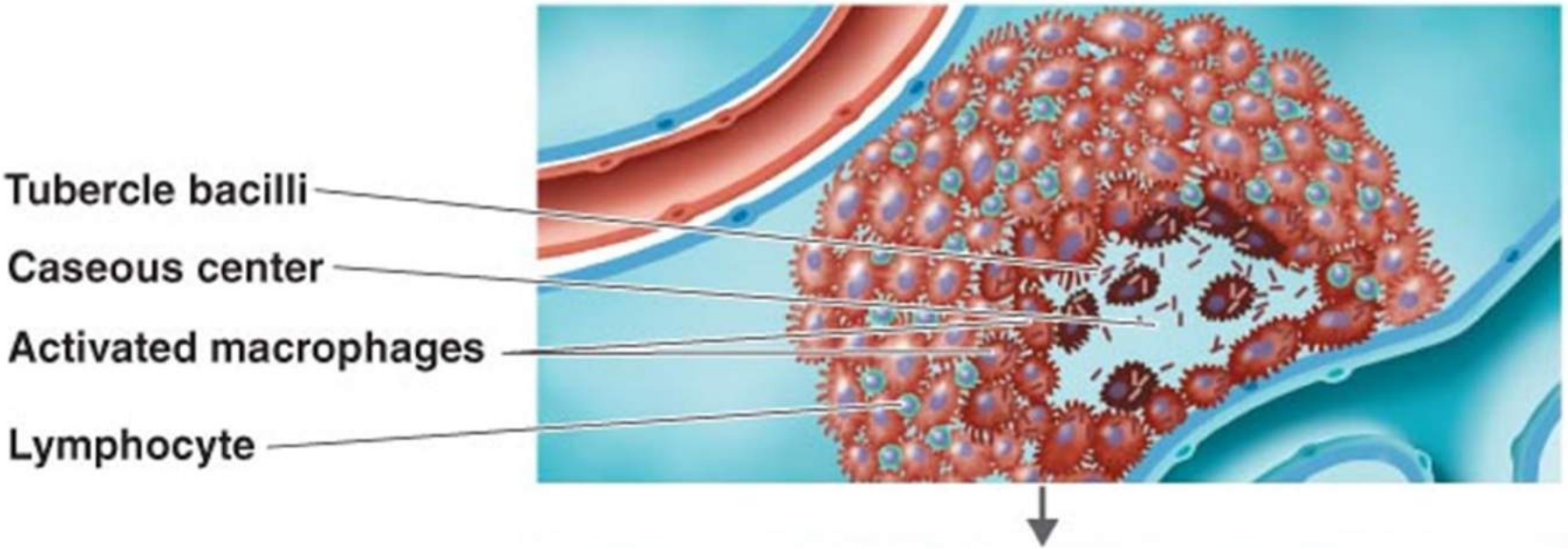
1 Tubercle bacilli that reach the alveoli of the lung (see Figure 24.2) are ingested by macrophages, but often some survive. Infection is present, but no symptoms of disease.

The Pathogenesis of Tuberculosis



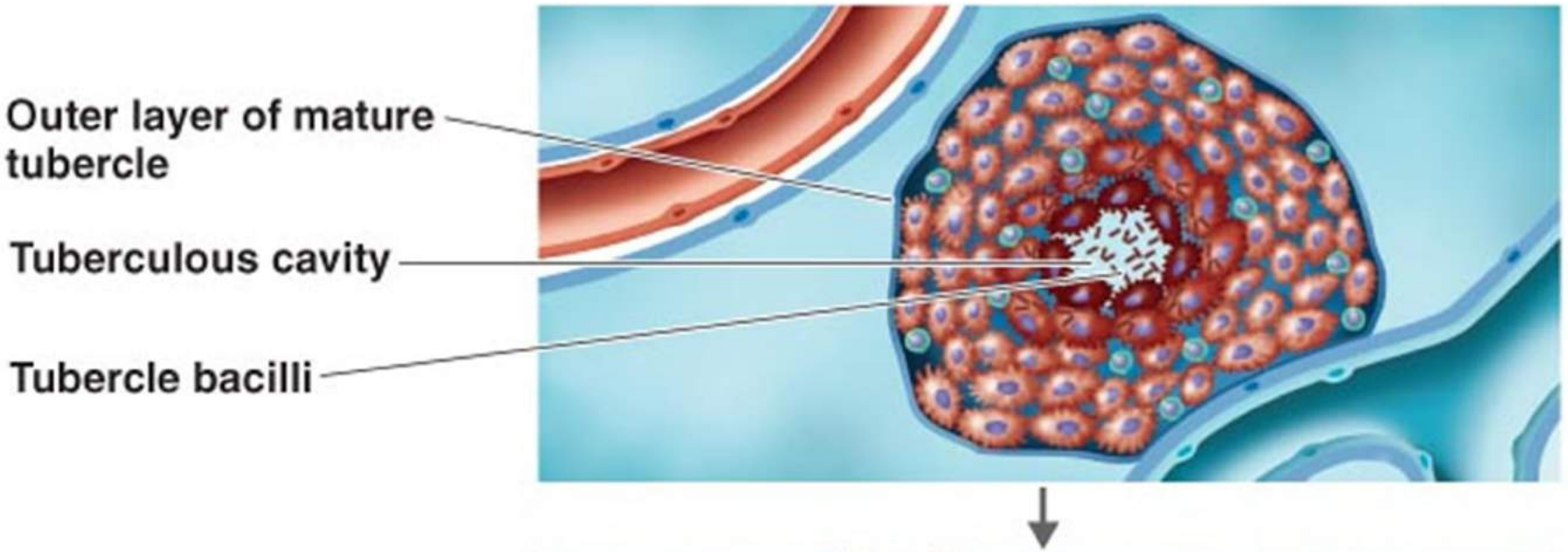
2 Tubercle bacilli multiplying in macrophages cause a chemotactic response that brings additional macrophages and other defensive cells to the area. These form a surrounding layer and, in turn, an early tubercle. Most of the surrounding macrophages are not successful in destroying bacteria but release enzymes and cytokines that cause a lung-damaging inflammation.

The Pathogenesis of Tuberculosis



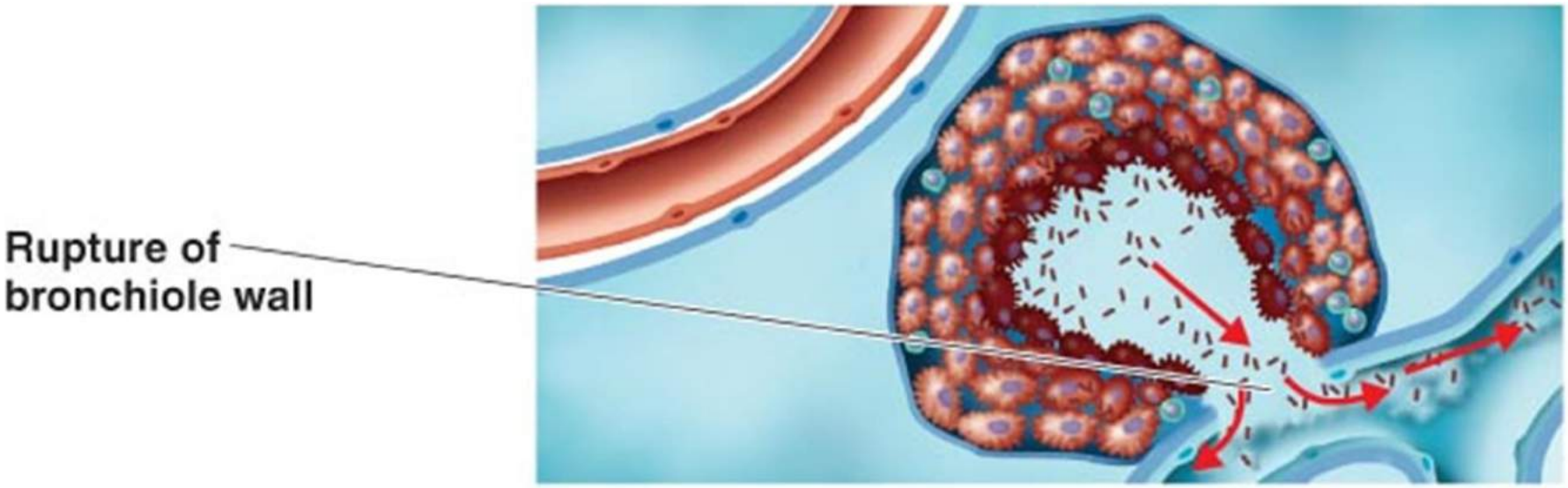
3 After a few weeks, disease symptoms appear as many of the macrophages die, releasing tubercle bacilli and forming a *caseous center* in the tubercle. The aerobic tubercle bacilli do not grow well in this location. However, many remain dormant (latent TB) and serve as a basis for later reactivation of the disease. The disease may be arrested at this stage, and the lesions become calcified.

The Pathogenesis of Tuberculosis



4 In some individuals, disease symptoms appear as a mature tubercle is formed. The disease progresses as the caseous center enlarges in the process called *liquefaction*. The caseous center now enlarges and forms an air-filled *tuberculous cavity* in which the aerobic bacilli multiply outside the macrophages.

The Pathogenesis of Tuberculosis



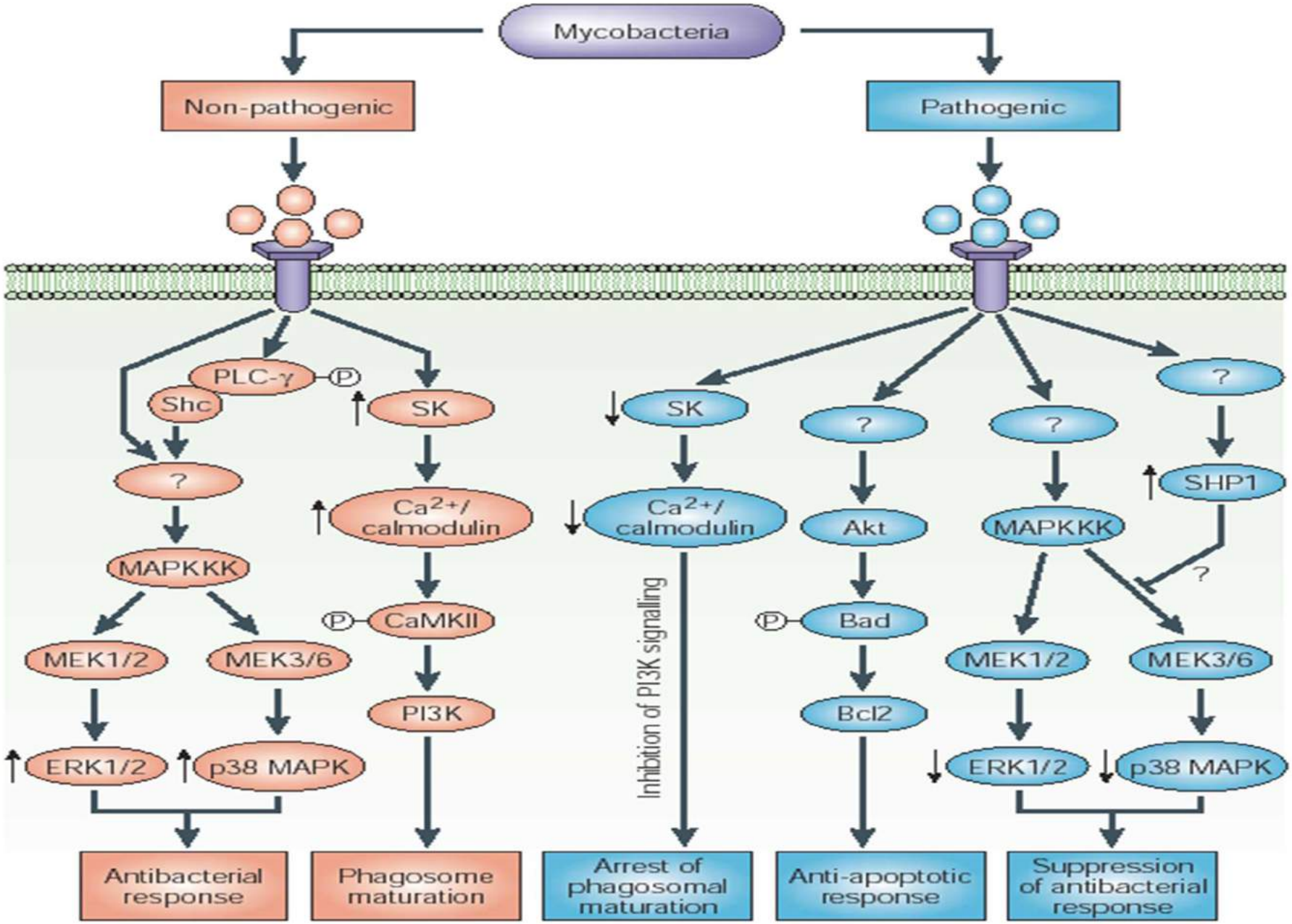
5 Liquefaction continues until the tubercle ruptures, allowing bacilli to spill into a bronchiole (see Figure 24.2) and thus be disseminated throughout the lungs and then to the circulatory and lymphatic systems.

Pathogenic Mycobacteria

- Survival inside macrophages.

- Intracellular signaling pathways altered:
  - Mitogen-activated protein kinases (MAPKs)
  - Interferon-gamma (IFN-γ)
  - Calcium pathways (Ca++)

- Host cell responses inhibited:
  - phagosome-lysosome fusion
  - apoptotic pathways
  - bactericidal immune response


Mycobacteria
Non-pathogenic
Pathogenic
PLC-γ
Shc
SK
?
Ca2+/ calmodulin
MAPKKK
CaMKII
MEK1/2
MEK3/6
PI3K
ERK1/2
p38 MAPK
Antibacterial response
Phagosome maturation
SK
Ca2+/ calmodulin
Inhibition of PI3K signalling
Arrest of phagosomal maturation
?
Akt
Bad
Bcl2
Anti-apoptotic response
?
MAPKKK
MEK1/2
MEK3/6
ERK1/2
p38 MAPK
Suppression of antibacterial response
?
SHP1
?

Phagosome maturation

Normal maturation events:

1. Phagocytosis of bacteria.

2. Acquire Rab5 (GTPase) and EEA1 (early endosome antigen 1) to direct fusion of phagosomes with early endosomes.

3. Late phagosomes lose Rab5 but acquire Rab7, along with LAMP proteins and cathepsin D (acid hydrolase) by fusing with lysosomes.

4. Vacuolar proton-ATPase molecules also acidify the phagolysosomes.

Inhibition of phagosome killing

- Mycobacteria secrete vesicles containing lipids and glycolipids that accumulate in endosomal/lysosomal organelles to inhibit phagosome maturation.

- LAM (lipoarabinommannan) is a cell wall glycolipid that can interfere with phagosome maturation and apoptosis.
  - Man-LAM: found in pathogenic mycobacteria.
  - Ara-LAM: found in non-pathogenic mycobacteria.
- Man-LAM suppresses the cytosolic Ca++ and calmodulin increase needed for PI3 and EEA1 recruitment that leads to phagosome maturation, as well as inhibiting delivery of acid hydrolases.

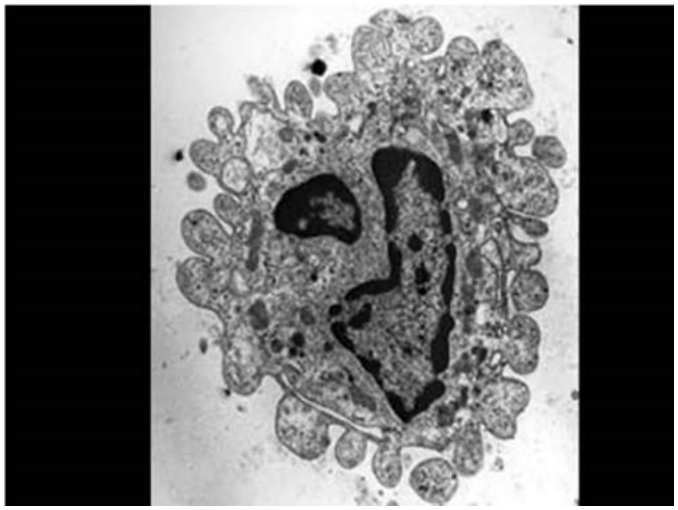
- TACO membrane proteins are associated with Mycobacteria-infected phagosomes and may also be involved with preventing lysosomal fusion.

- Mycobacteria survive in phagosomes that have not fused with lysosomes and so are not acidified.

Apoptosis = programmed cell death

Extrinsic pathway – Extracellular ligands (TNF-α, FasL, etc.) bind to death receptors on cell membrane.

Intrinsic pathway – Intracellular translocation of cytochrome c from mitochondria to cytosol.



- Both pathways lead to a caspase cascade, DNA degradation, production of apoptotic bodies, and
- antigen presentation.

Inhibition of apoptosis

Mycobacterial Man-LAM:

-prevents the Ca++ increase that would increase mitochondrial permeability and cytochrome c release.

-activates the Akt cascade that phosphorylates Bad to keep it from binding Bcl-2. Free Bcl-2 inhibits cytochrome c release and inhibits caspase activity.

IL-10 production:

- releases TNFR2 to block TNF-α activity that would activate the death receptor and external apoptotic cascade.

Innate Immune Response

Normal events:

1. Bacteria enter host cells

2. Intracellular signaling cascades:
   - MAPK (JNKs, ERKs, p38 MAPKs), JAK/STAT

3. Cytokines released:
   - IL-1, IL-6, IL-12, TNF-α, IFN-γ

4. Increased tissue permeability and recruitment of inflammatory cells to kill bacteria.

Inhibition of innate immunity

MAPK pathway:

Less virulent Mycobacteria induce a sustained p38 signal cascade and immune response.

More virulent Mycobacteria prevent sustained activation of p38 and ERK signal cascades.

JAK/STAT:

Virulent Mycobacteria may cause a reduction of IFN-γ receptors that inhibit the JAK/STAT cascade.

Mycobacterial lipids may induce SOCS (*Suppressors of cytokine signalling*) expression and inhibit the JAK/STAT signaling.

Adaptive Immune Response

Normal events:

- Dendritic cells phagocytose bacteria and present antigen for T cell differentiation:
  - T helper-1 cells: IFN-γ for intracellular pathogens.
  - T helper-2 cells: IL-4 for extracellular pathogens.

- Toll-like receptors (TLRs) and C-type lectins on dendritic cells sense pathogens.

Inhibition of adaptive immunity

Man-LAM binds the C-type lectin DC-SIGN:

inhibits dendritic cell maturation and T-cell activation.

induces secretion of IL-10 to inhibit activated dendritic cells (adaptive immune response) and macrophages (innate immune response), as well as inhibiting production of inflammatory cytokines IL-12 and TNF-α.

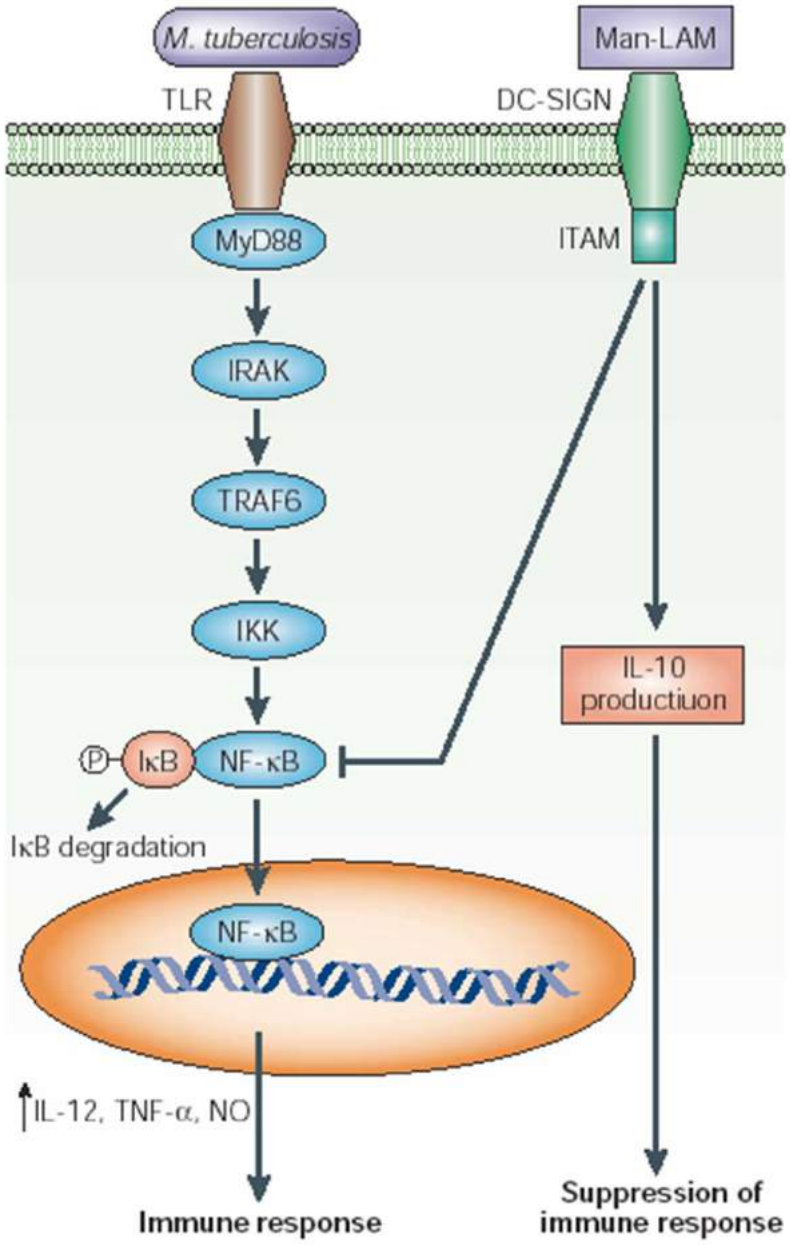


Figure 6 | **Disruption of dendritic-cell signalling pathways**

ITAM: Immunoreceptor Tyrosine-Based activation Motif

DC-SIGN: dendritic cell-specific ICAM-3 grabbing nonintegrin

Treatment of Tuberculosis

- **Treatment**: Prolonged treatment with multiple antibiotics
- **Vaccines**: BCG, live, avirulent *M. bovis*; not widely used in United States.

- Bacille Calmette Guerin (BCG) is the current vaccine for tuberculosis. It was first used in 1921. BCG is the only vaccine available today for protection against tuberculosis. It is most effective in protecting children from the disease.
- BCG contains a live attenuated (weakened) strain of Mycobacterium bovis.

Novel drugs needed

Current TB treatment:

Multiple drugs used.

6-9 month treatment.

Multi-drug resistance.

Drugs target actively replicating bacteria but persistent infections occur.

Drug development

1. Identify target proteins using mutant studies.
2. Use purified enzyme to screen compound libraries. Consider compound activity, cellular permeability, solubility, stability.
3. Test compounds in vitro for MIC, toxicity profiling, and selectivity profiling.
4. Next test them in Mycobacteria-infected macrophages and evaluate pharmacokinetic properties to identify 'lead compounds' for in vivo testing in mice and clinical trials.

Novel drug targets

Mycobacterial targets:

- Kinases that phosphorylate host proteins.
- Phosphatases that dephosphorylate host proteins.
- Other enzymes (isocitrate lyase, synthases)

Borrow concepts from cancer and diabetes research for drug candidates.

Host cell targets:

- Can we activate signaling systems that Mycobacteria act to suppress to promote a bactericidal response?

Acknowledgement and Suggested Readings:

1. Medical Microbiology, A guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Investigation and Control; Barber, Irving, Swann and Perera; Elsevier Publication
2. Microbiology, An Introduction; Tortora, Funke and Case; Pearson Publication
3. Microbiology; Prescott, Harley and Klein; The MacGraw-Hill Companies
4. Microbiology: Principles and Explorations; Jacquelyn G Black; John Wiley and Sons Inc.
5. Brock Biology of Microorganisms; Madigan, Martinko, Stahl and Clark; Benjamin Cummings (Pearson Publication)

Thanks