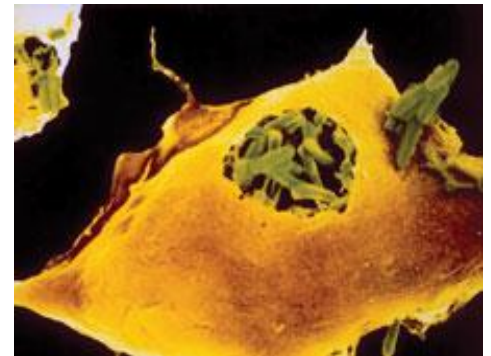


# An overview of mycobacteria

Dr. Nitya Krishnan



## Learning outcomes

- Give examples of the different species of mycobacteria
- Compare and contrast the characteristics of slow growing and fast growing mycobacteria
- Outline the life cycle of *M.tuberculosis*

# Overview

- Classification of mycobacteria
- Identification of mycobacteria in the laboratory
- Mycobacteria and human disease
- Pathogens: *M. tuberculosis* and *M. leprae*
- ‘Environmental’ mycobacteria and disease
- Animal disease and mycobacteria

# Medically important mycobacteria

## Obligate pathogens

- *M. tuberculosis* complex
- *M. leprae*

## Environmental or 'Atypical' mycobacteria

(>40 species)

- *M. avium* complex
- *M. ulcerans*
- *M. kansasii*
- *M. marinum*

## Rapid growers:

- *M. chelonae*
- *M. fortuitum*

# The *M. tuberculosis* complex

<i>M. tuberculosis</i>	<i>M. africanum</i>	<i>M. cannetti</i>	<i>M. bovis</i>	<i>M. microti</i>
humans	humans (West Africa)	humans (rare)	cattle deer badgers humans goats seals lions llamas . . .	voles mice ferrets shrews

# Mycobacteria and human disease

<b>Disease in humans</b>	<b>Mycobacterial species</b>
Tuberculosis	<i>M.tuberculosis</i> complex
Leprosy	<i>M.leprae</i>
Buruli ulcer	<i>M.ulcerans</i>
Swimming-pool granuloma	<i>M.marinum</i>
Post-traumatic abscesses	<i>M.fortuitum/M.chelonae</i>
Lymphadenitis (usually children)	<i>M.avium</i> complex
Opportunistic lung disease	<i>M.avium</i> complex, <i>M.kansasii</i>
HIV-associated disseminated disease	<i>M.avium</i> complex

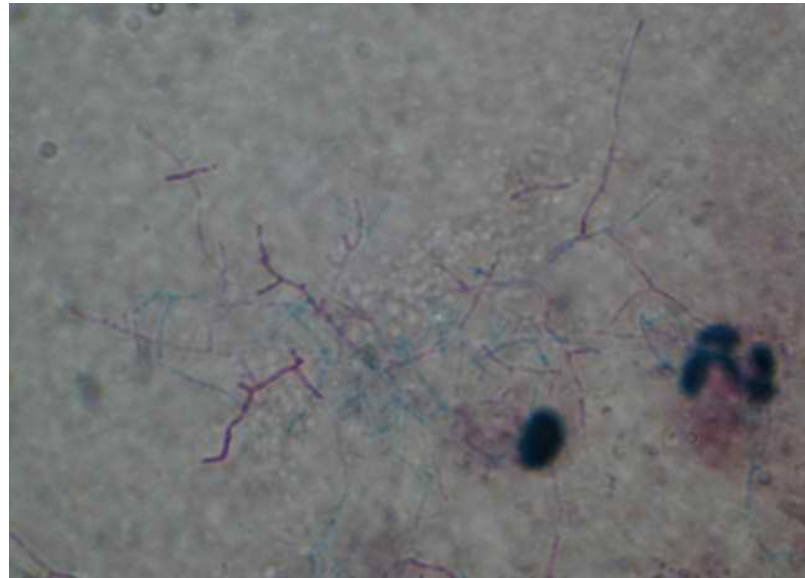
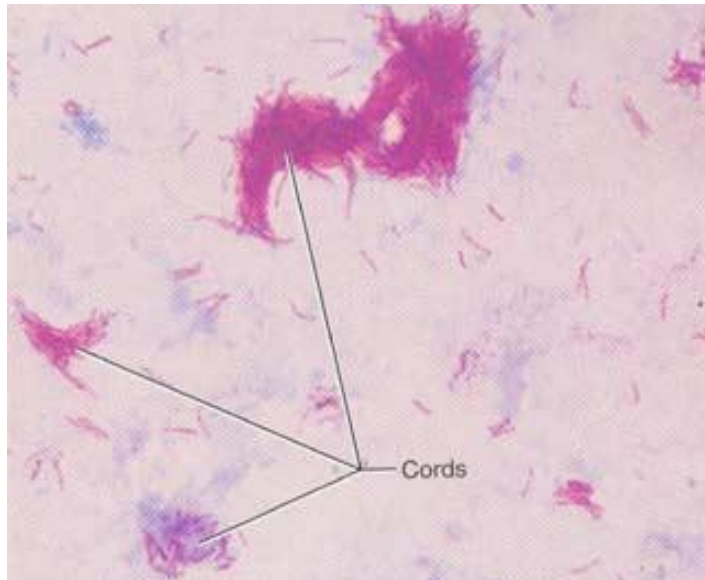
# Defining 'Clinical' laboratory features of mycobacteria

- Aerobic/microaerophilic, non-motile bacilli
- Weakly gram positive
- 'Acid fast'
- Slow growing and fastidious: many take ~4 weeks for visible growth on special media e.g. Lowenstein-Jensen media
- Photochromogens (e.g. *M.kansasii*), scotochromogens (e.g. *M.gordoniae*) and non-chromogens (*M.tuberculosis*)



# What does 'Acid-fast' mean ?

- Property discovered by Paul Ehrlich in 1883
- Adapted by Ziehl and Nielsen: the 'ZN' stain (1884)
- Definitive characteristic of all mycobacteria
- Other genus can be variably 'acid-fast': *nocardia* (branching)
- Ability to resist decolourisation with acidified alcohol when stained with an arylmethane stain (e.g. carbol-fuchsin)

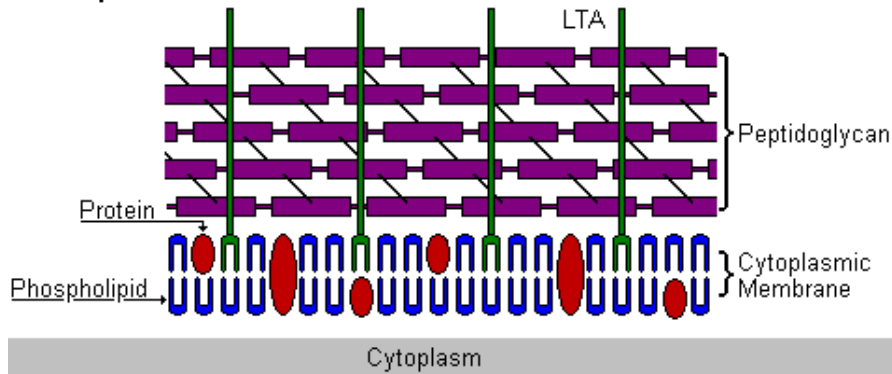




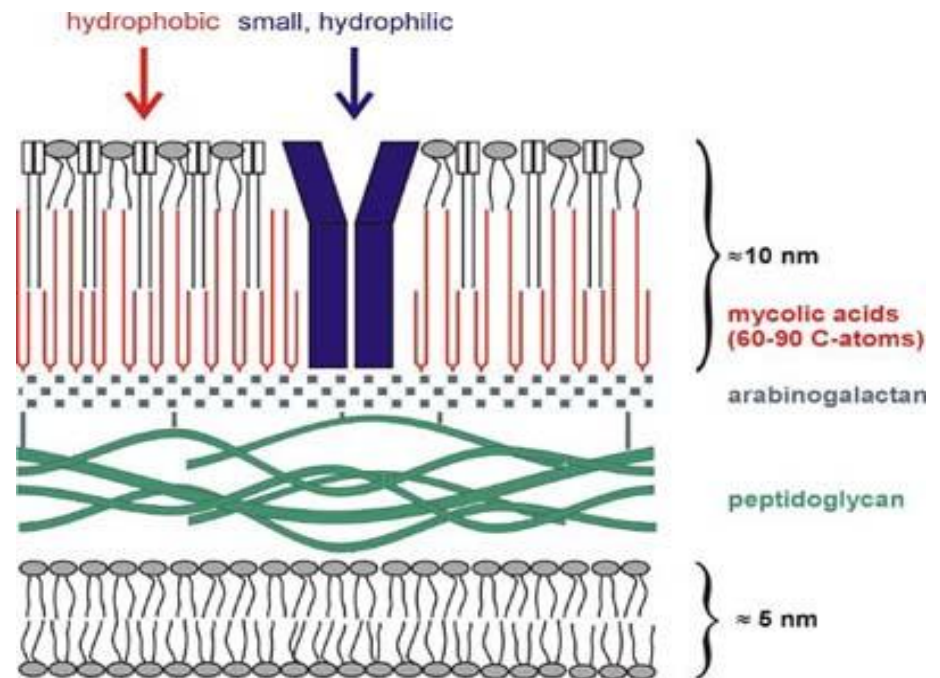
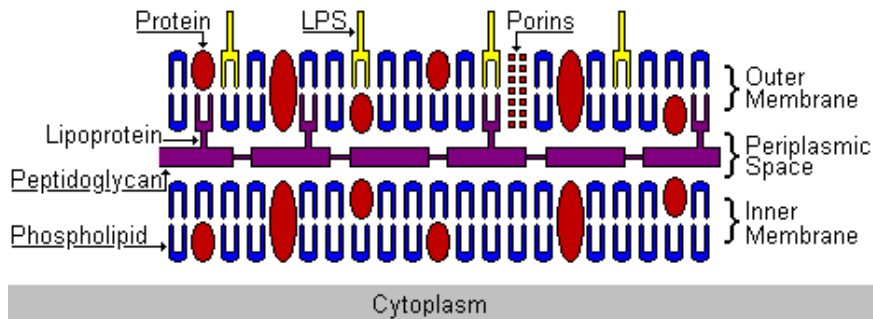
# Why are mycobacteria acid fast?

cell wall

## Gram-positive Cell Wall



## Gram-negative Cell Wall

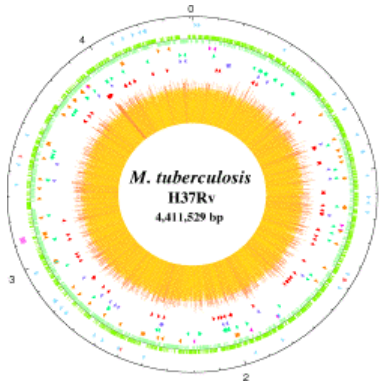
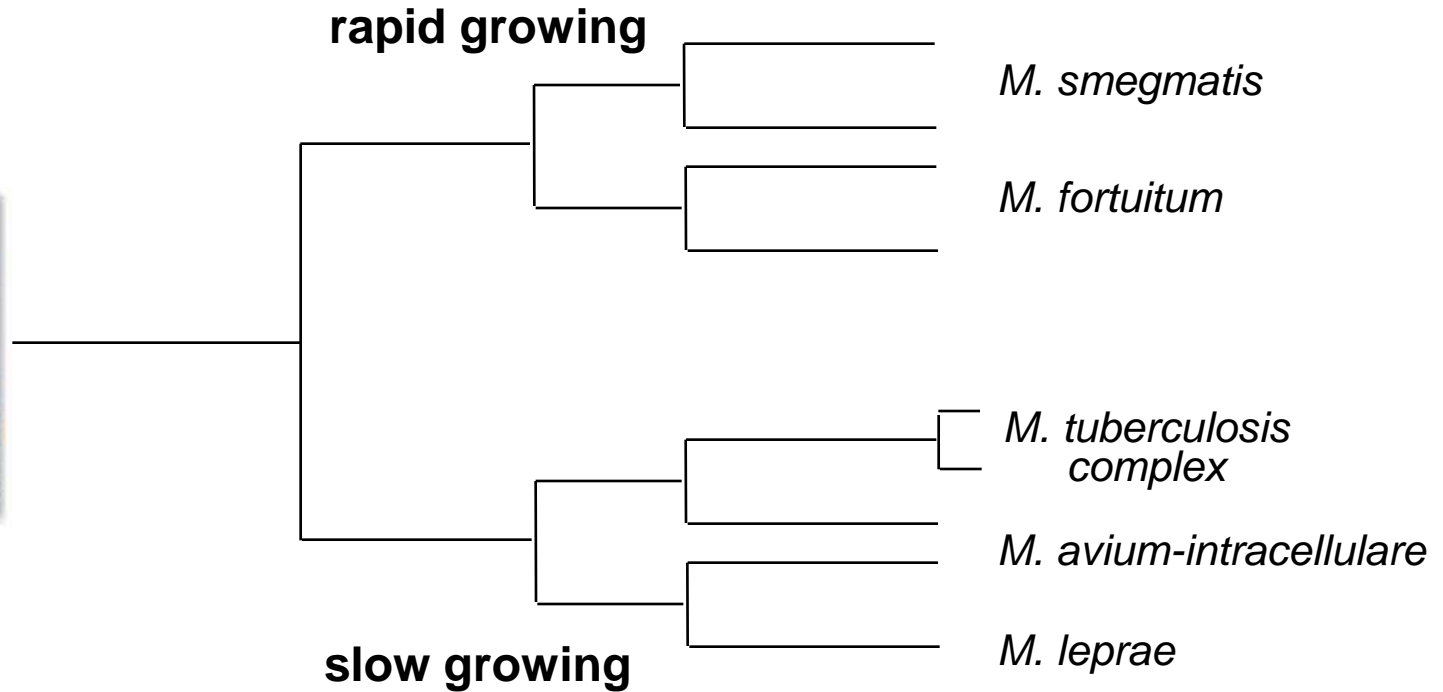


Cell wall of mycobacteria

# Mycobacterial cell wall

- High lipid content: extremely hydrophobic
- Ability to survive in the environment
- Ability to resist complement lysis
- Ability to resist antibiotics (impermeable)

# Mycobacterial genomics



## Genome size

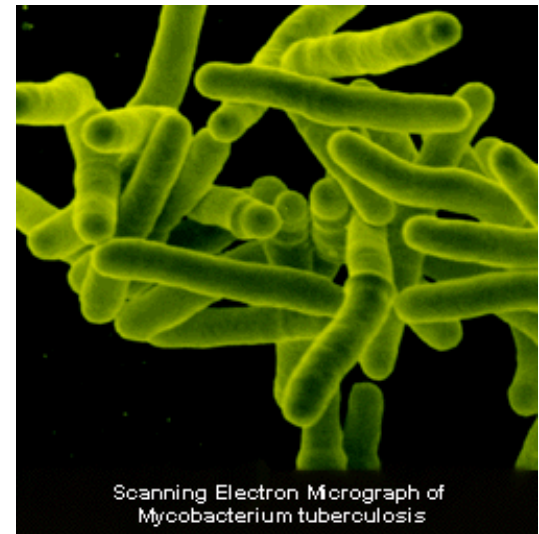
<i>M. smegmatis</i>	~7 Mb
<i>M. tuberculosis</i>	4.41 Mb
<i>M. leprae</i>	3.27 Mb

*M. tuberculosis* genome. Cole et al. 1998. Nature 393, 537-544

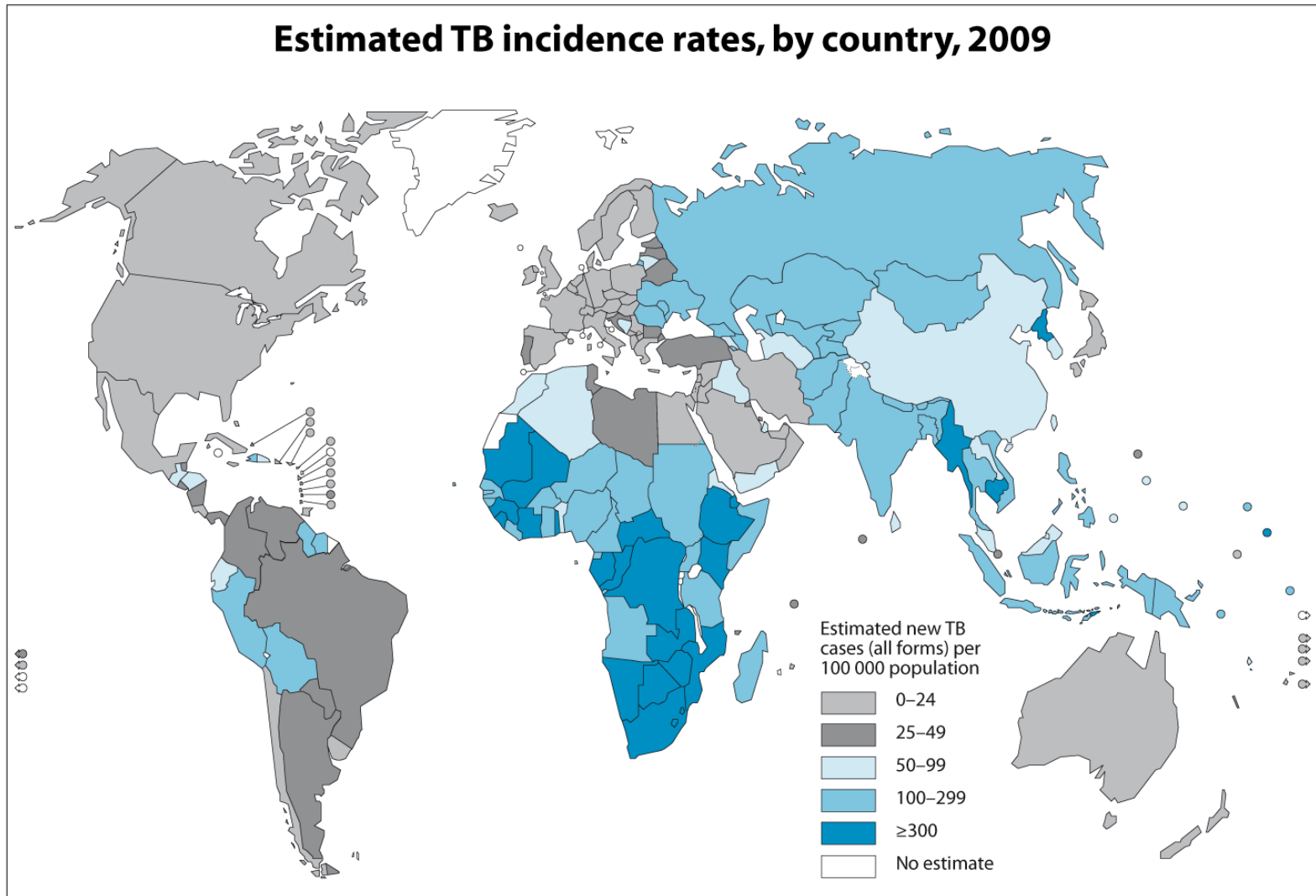
# Mycobacterial genomics

- Whole genome sequence of *M.tb*, *M.bovis*, and *M.leprae* known
- High G+C content (65.6% *M.tb*)
- *M.tb* genome: 4,411,532 bp. Single circular chromosome (similar size to *E.coli*)
- Around 4000 genes; function known of ~50%
- Large number of genes involved in lipid metabolism
- Complex gene regulatory system: has to switch between aerobic and microaerophilic conditions

# ***Mycobacterium tuberculosis (M.tb)***

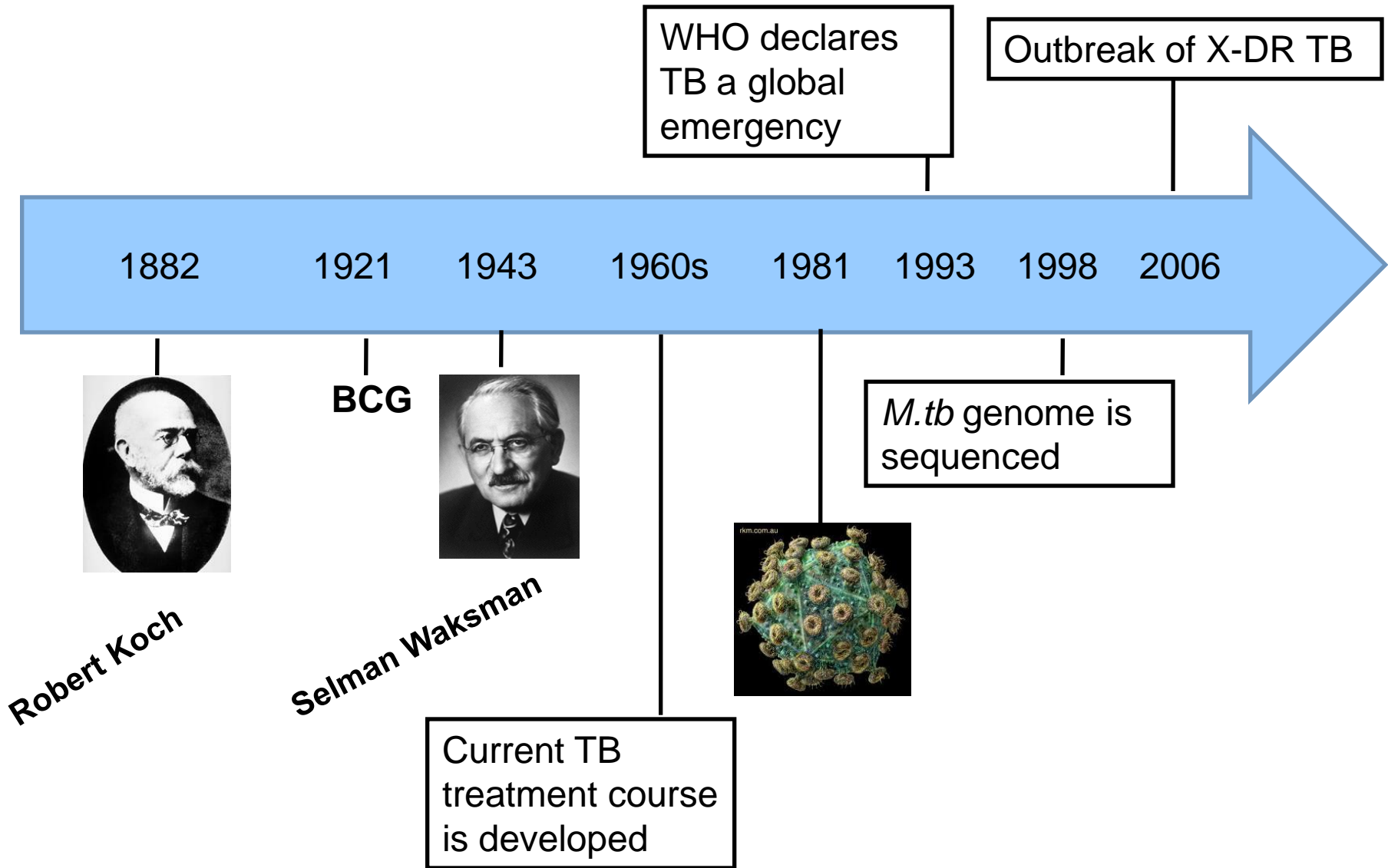


# TB Global Burden



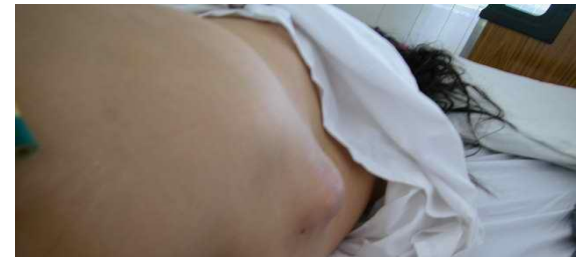
- 1/3<sup>rd</sup> of the world's population infected
- In 2009, 1.7 million people died from TB
- There are 3,500 cases of TB in London each year

# Timeline - TB



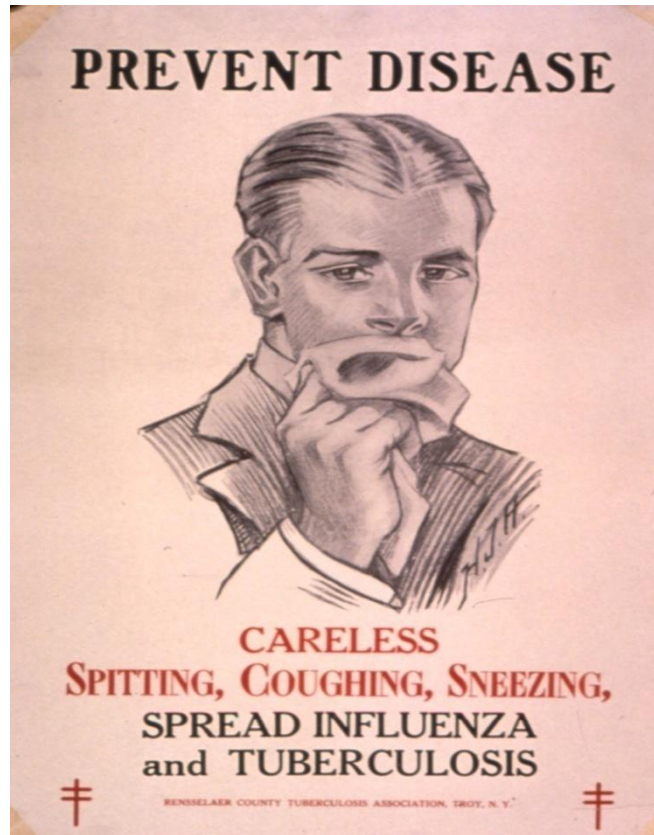
# Tuberculosis: the disease

- 80% lung disease
- 20% extra-pulmonary disease
  - Lymph node
  - Brain
  - Bone
  - Kidney



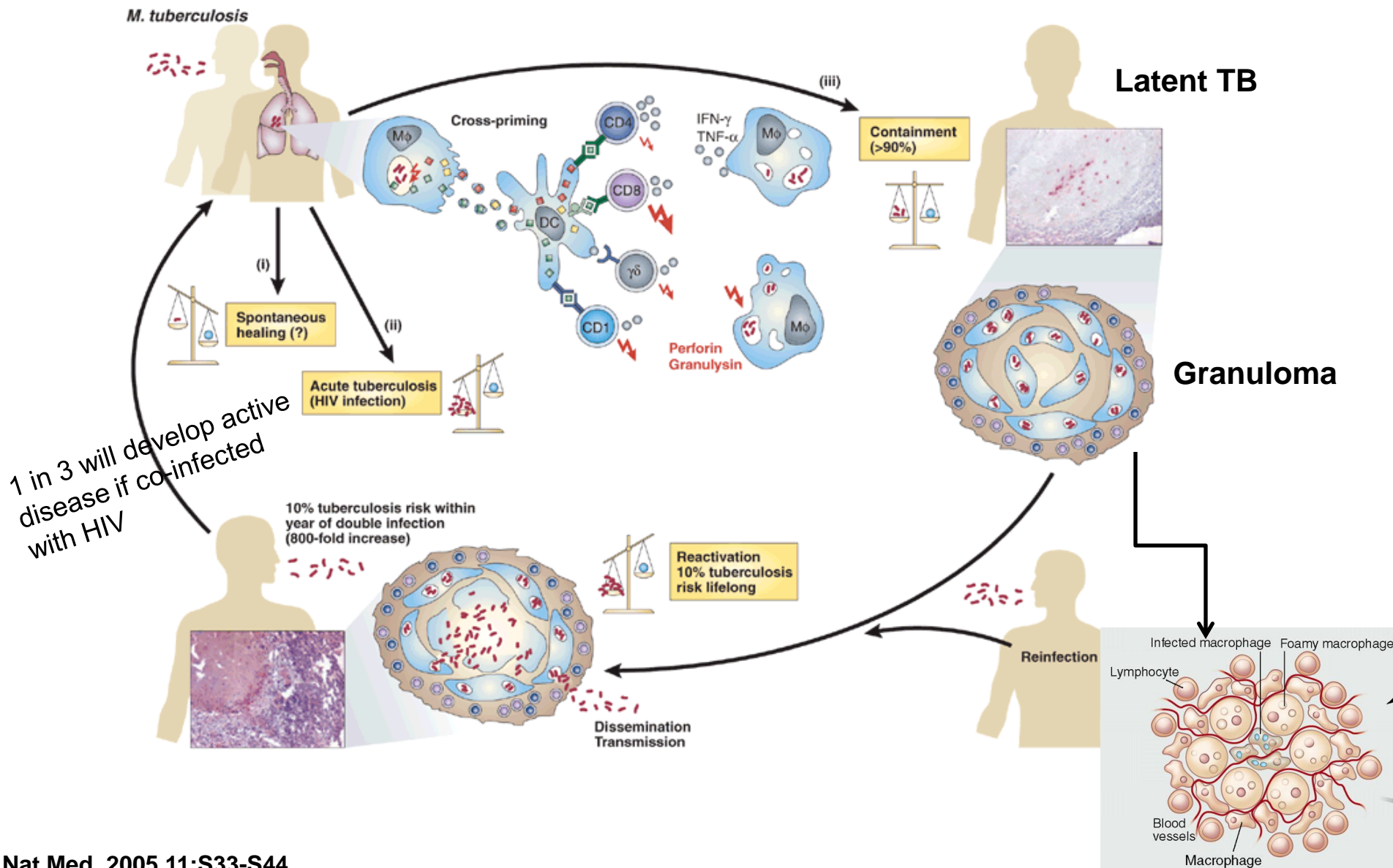


# Transmission of *M.tb*

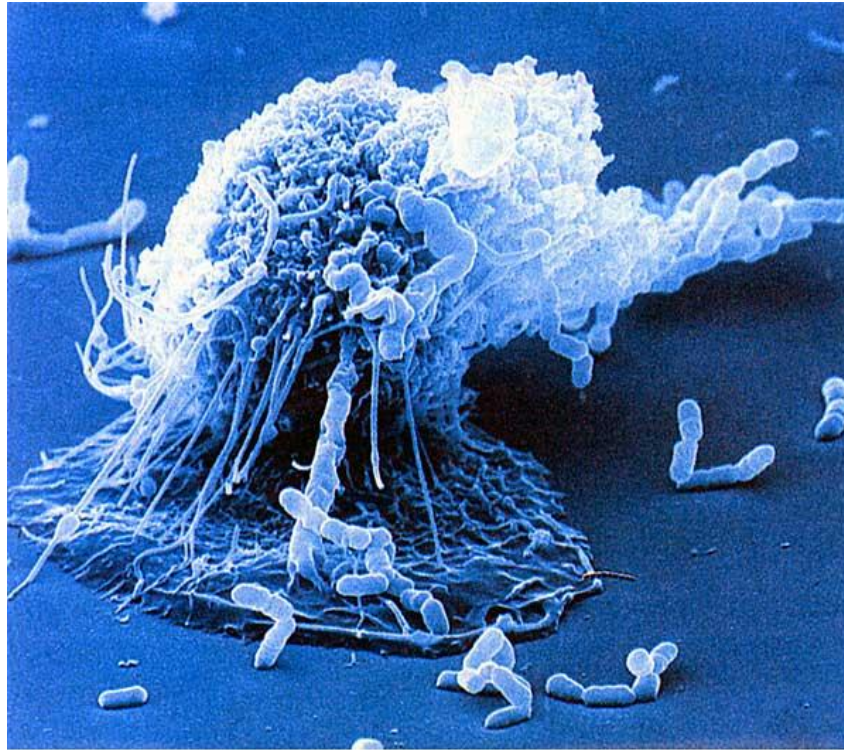


Rensselaer County Tuberculosis Association in Troy, NY. Poster dated- 1925

# Life cycle of *M.tb*

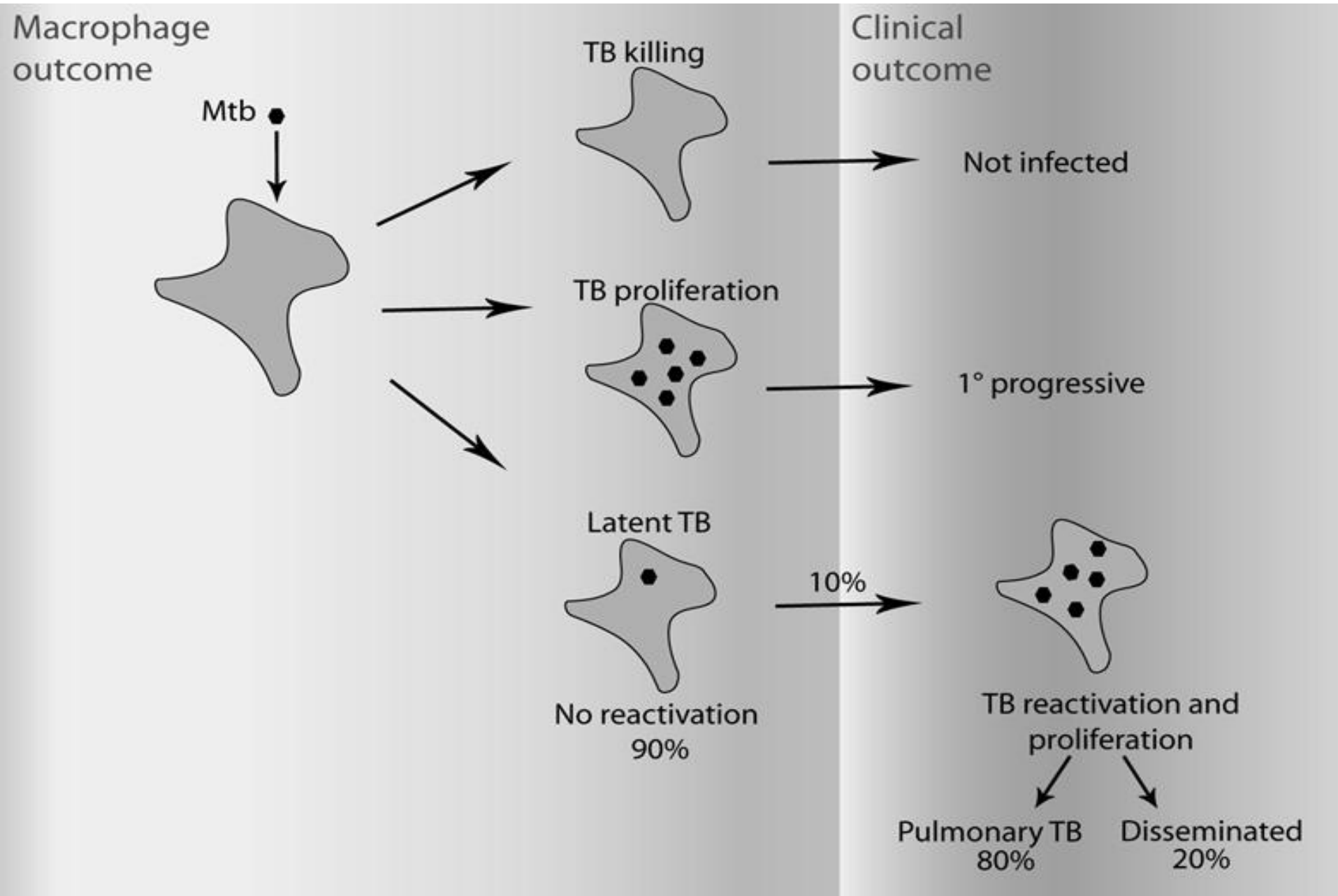


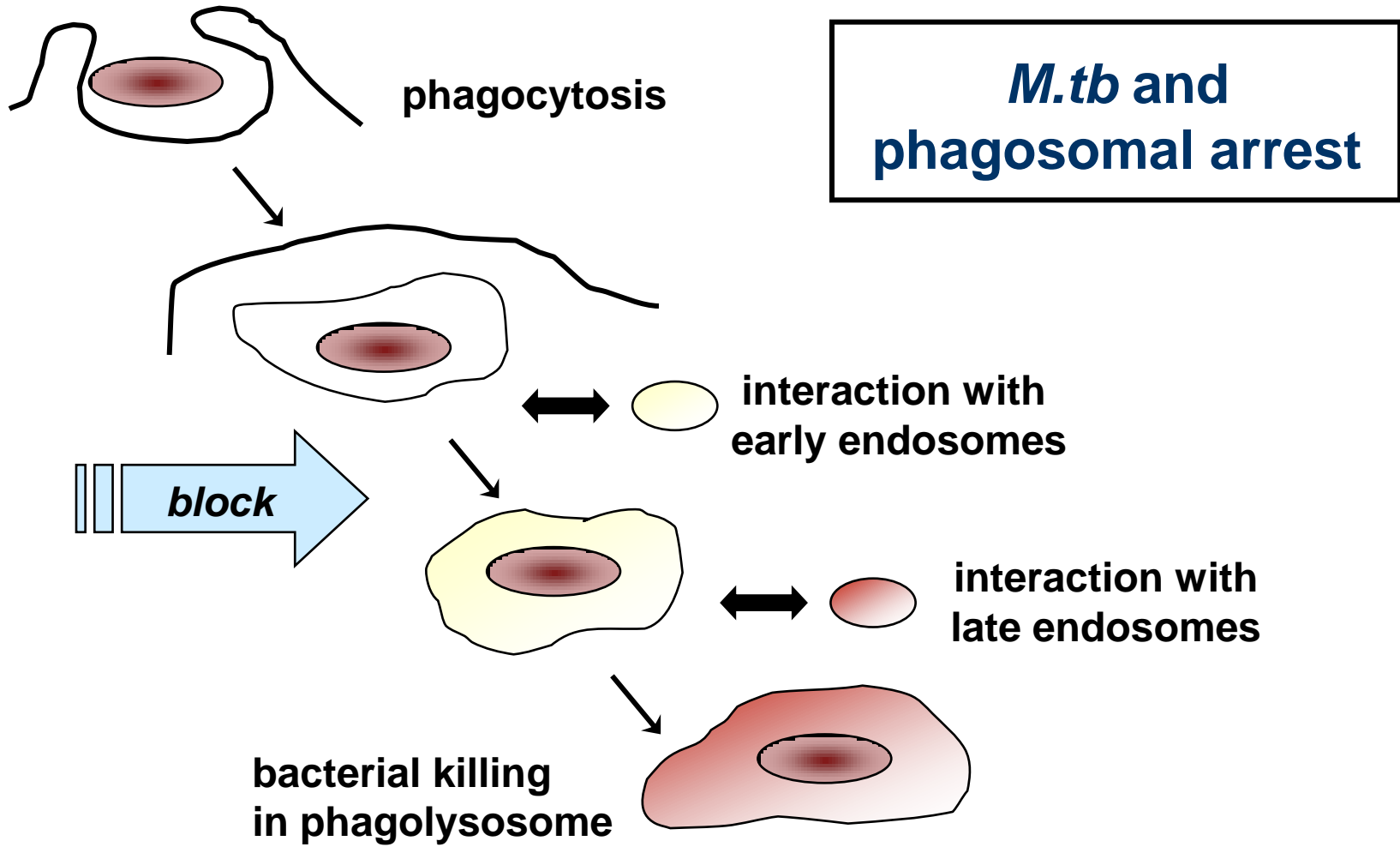
# Host-pathogen interaction



***M.tb* and macrophages**

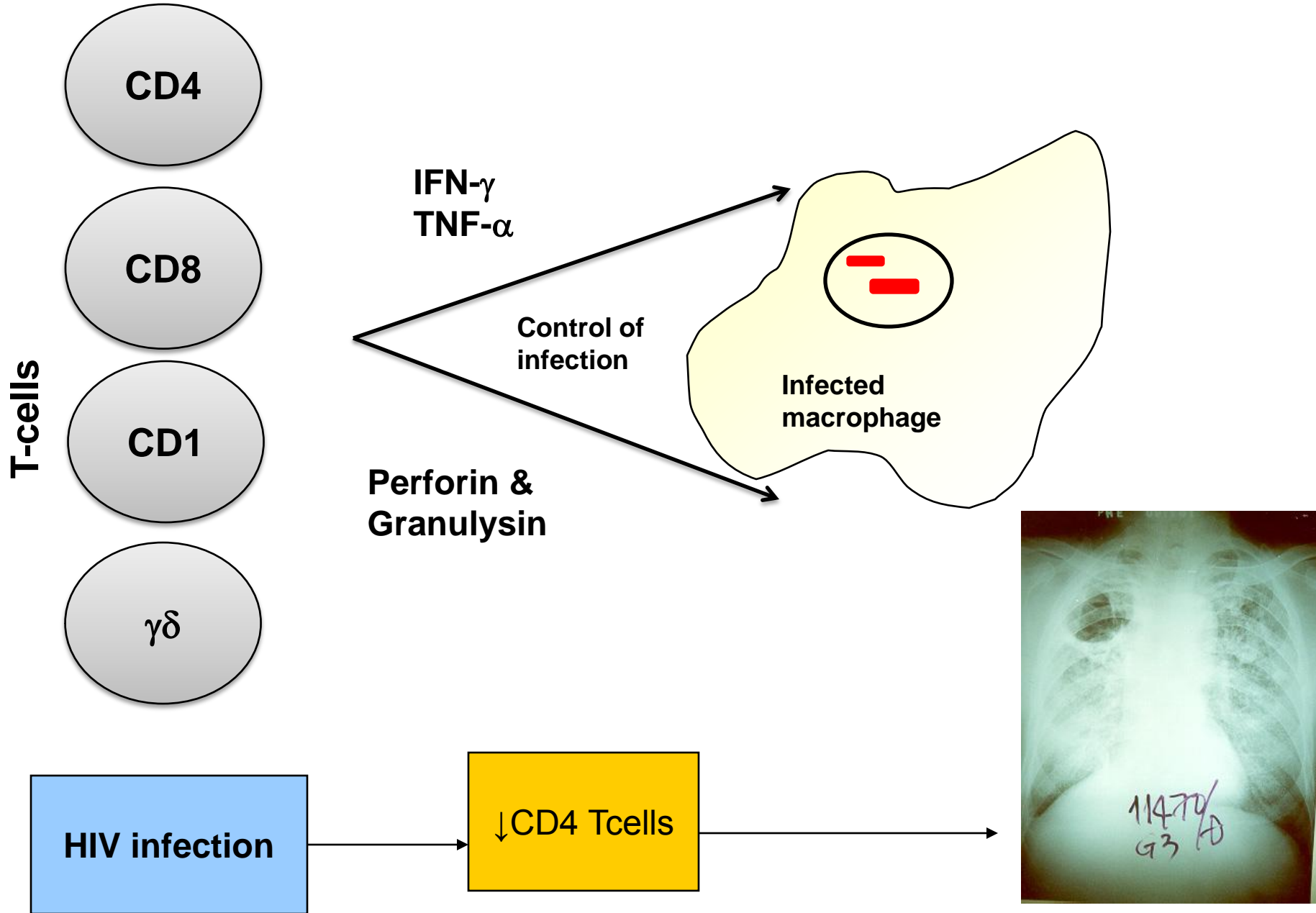
# M.tb and macrophages



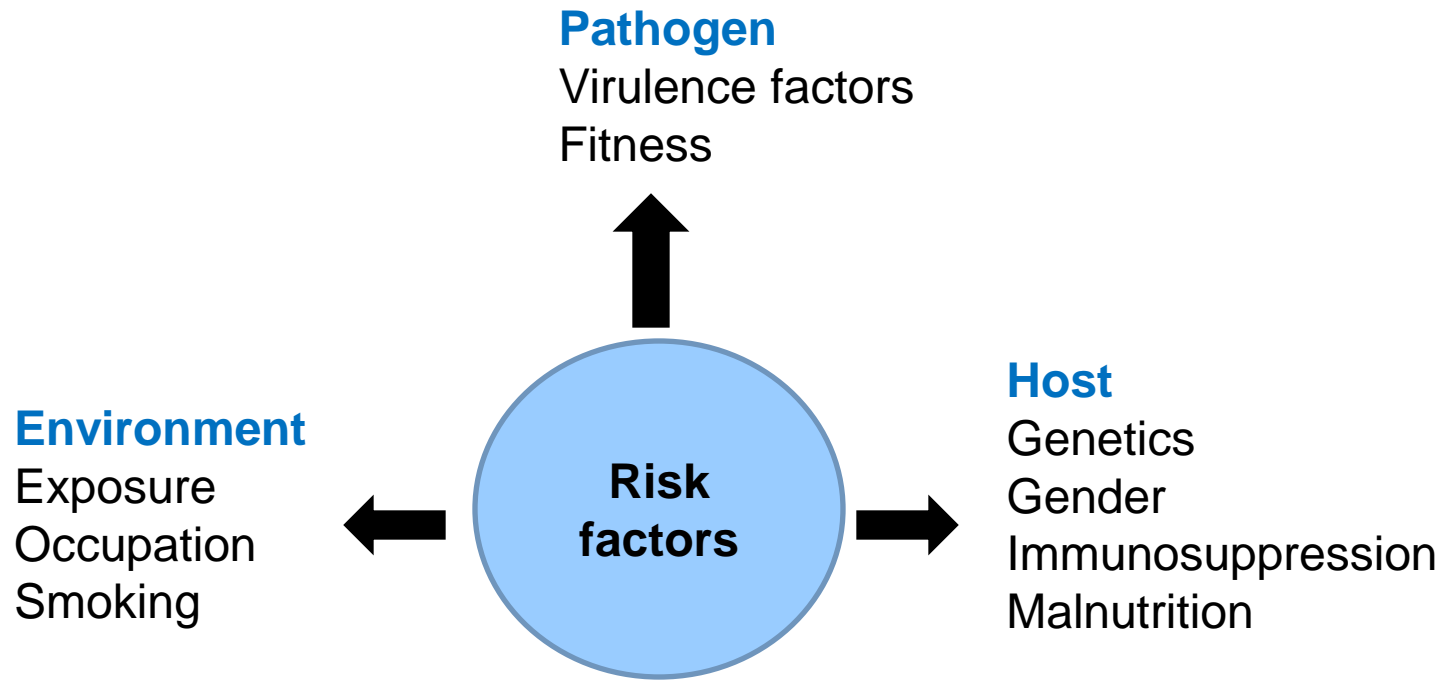


Russell DG. Who put the tubercle in tuberculosis?  
Nat Rev Microbiol 2007,5:39-47.

# T-cell mediated immunity



# TB risk factors



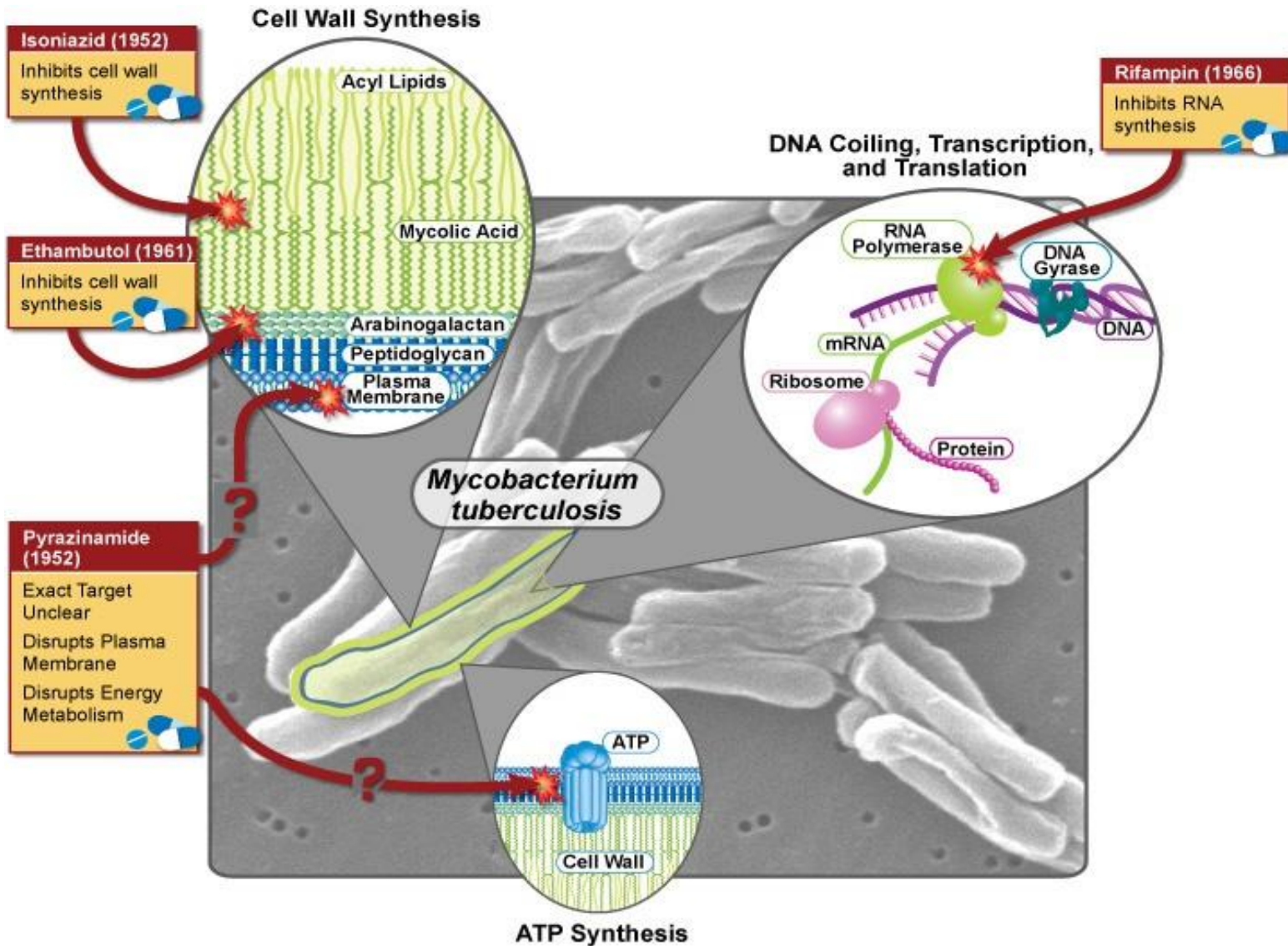
## Single gene defect- IFN- $\gamma$

- Maltese family
- Disseminated mycobacterial infection
- Failure to produce TNF- $\alpha$  and up-regulate IFN- $\gamma$
- Polymorphism in the IFN- $\gamma$  receptor
- Selective immune-suppression

MJ Newport, CM Huxley and S Huston *et al.*, A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection, *N Engl J Med* **335** (1996), pp. 1941–1949



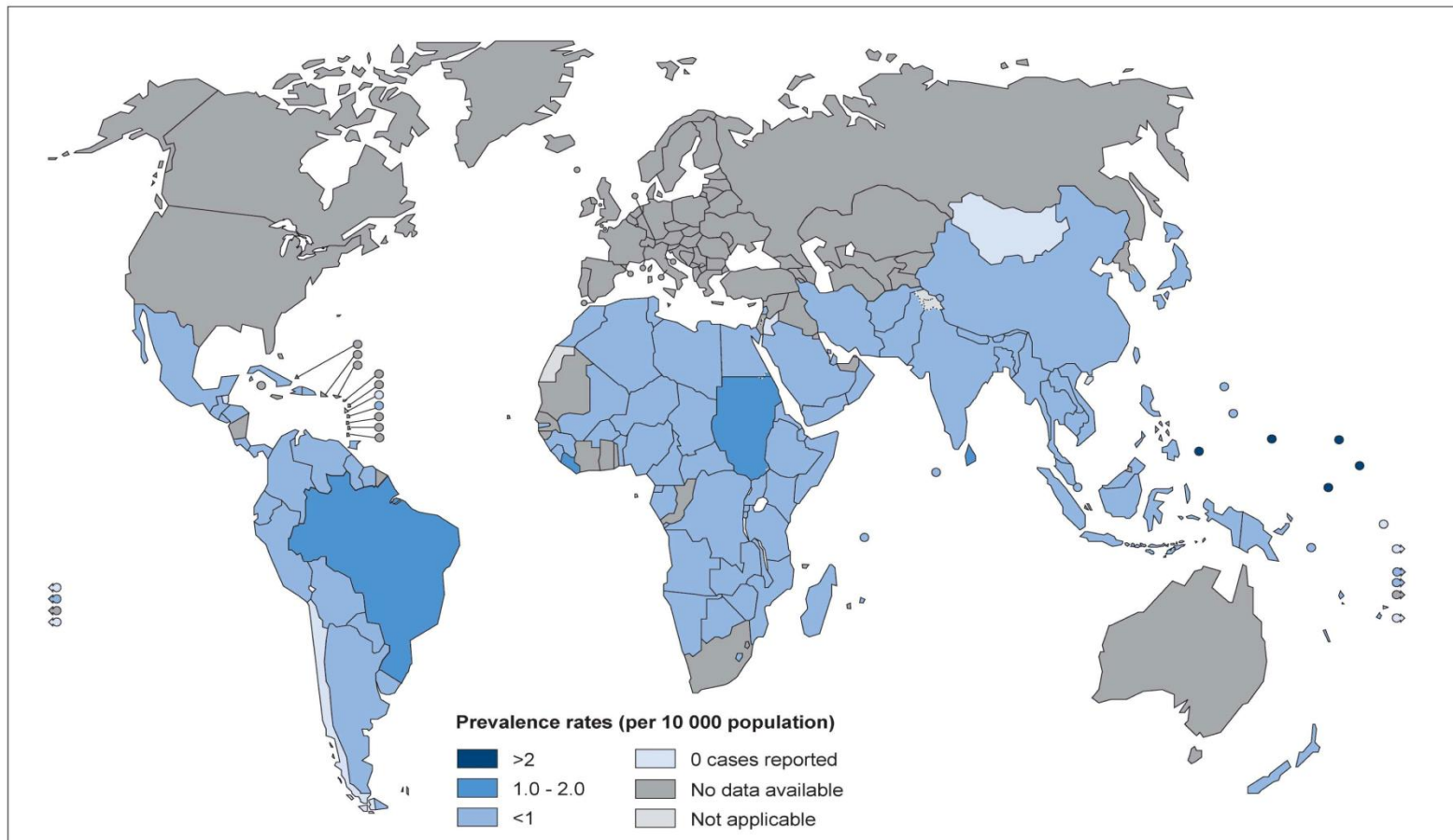
# Treatment: anti-TB drugs



# Leprosy and *M.leprae*

- Second most prevalent mycobacterial disease worldwide
- 228,474 new cases during 2010 (decreasing)

Leprosy prevalence rates, data reported to WHO as of beginning January 2011

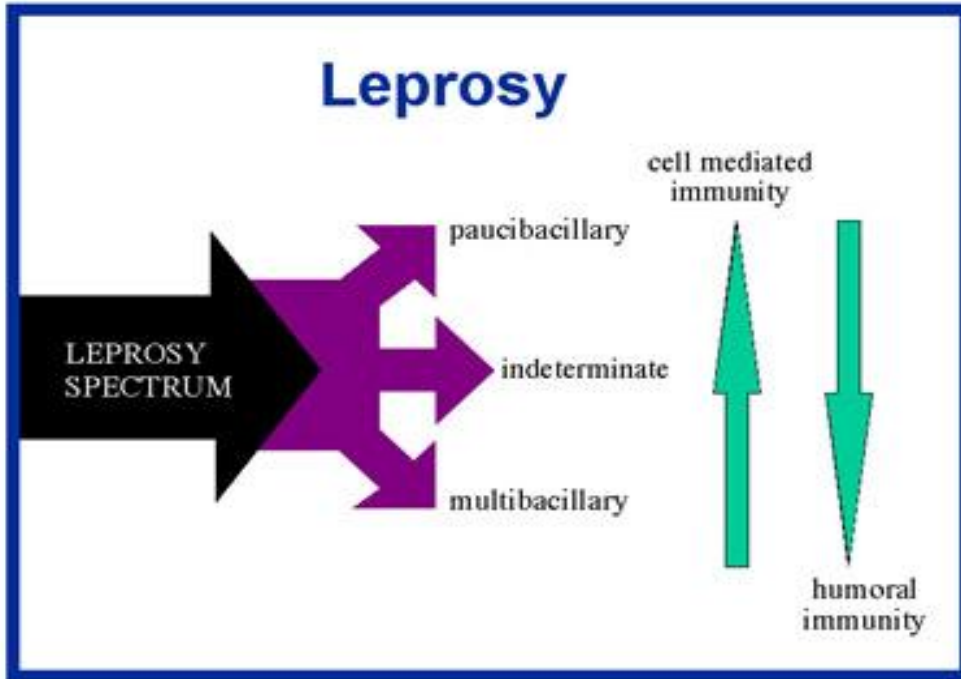


## *M. leprae*



- Only culturable in the 9 banded armadillo
- 12-day replication time *in vivo*
- Diagnosed by demonstrating acid-fast bacilli in skin biopsies

# Leprosy



# Leprosy: transmission and treatment

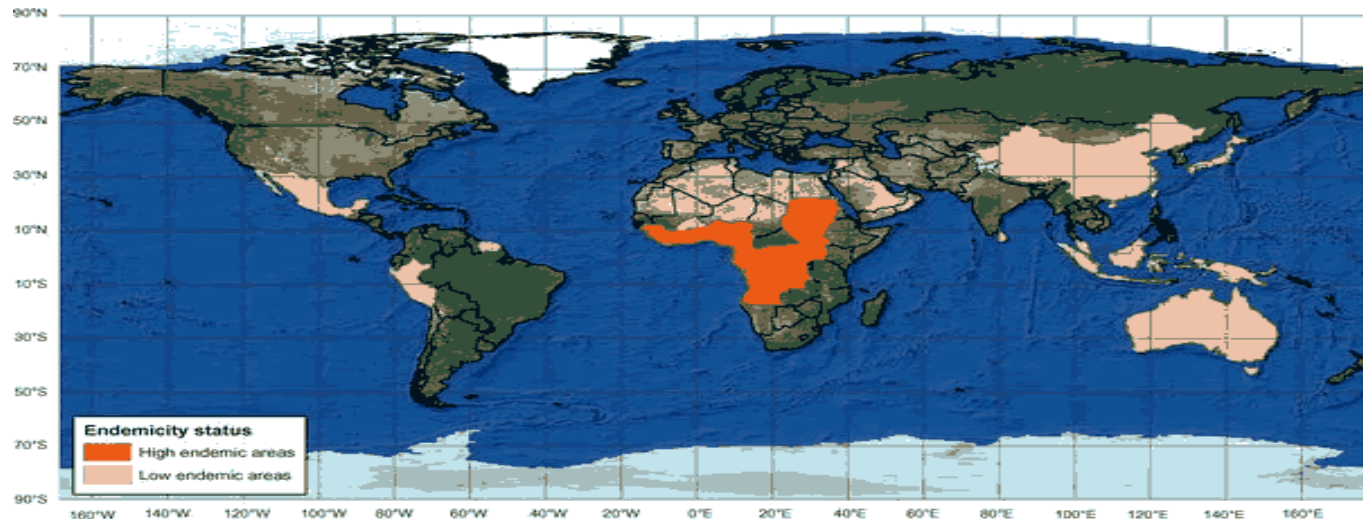


- Transmitted by nasal discharges
- Low infectivity (compared with *M.tuberculosis*)
- Incubation period about 5 years
- Treatment: rifampicin, clofazimine, dapsone. 6-12 months. Highly effective.

# Buruli ulcer: *M.ulcerans*



- Environmental bacteria – may live within small aquatic organisms
- Transmission unknown
- Culturable at 27-30°C
- Virulence dependent on production of toxic mycolactone – tissue destruction
- Treatment: drugs (rifampicin and streptomycin) and surgery



Mycolactone suppresses T cell responsiveness by altering both early signaling and posttranslational events.  
The Journal of Immunology 2010. 184: 1436-1444

# Swimming pool granuloma

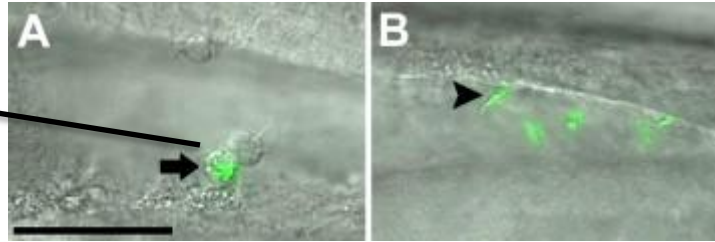
## *M. marinum*



- Environmental bacteria
- Photochromagen
- Drug treatment may take 18 months
- Similar traumatic abscesses can be caused by the rapid growing mycobacteria: *M. fortuitum* & *M. chelonae*

# Understanding the pathogenesis of mycobacteria using *M. marinum* infected zebrafish embryos

*M. marinum* labelled with green fluorescent protein



Infected macrophage in a zebrafish embryo

Extracellular bacteria in the hindbrain

**Zebrafish-*Mycobacterium marinum* model for mycobacterial pathogenesis**

FEMS Microbiology Letters 2003. 225: 177-182

## ***M. marinum***

- Close genetic relative of *M. tb*
- Replicates in host macrophages
- BSL-2 organism
- Faster generation time

## **Zebrafish**

- Embryos aid in understanding the early events of mycobacterial pathogenesis (contribution of the innate immune response)
- Embryos are transparent and hence allows for the real-time monitoring of *in vivo* events
- Identification of host genes that modulate TB susceptibility



## *M.avium* complex

- *M.avium* and *M.intracellulare*
- Slow growing non-chromagen
- Environmental organism
- Lymphadenitis in immune-competent children
- Lung disease in adults with co-existing lung disease
- Disseminated disease in advanced HIV disease



# Mycobacteria and animal disease

- *M. bovis*: tuberculosis of cattle and other animals (e.g. badgers)



- *M. paratuberculosis*: Johne's disease of cattle (fatal gastroenteritis)
- *M. avium*: avian tuberculosis and lymphadenitis in deer and pigs

# Summary

- Mycobacteria are some of the most important pathogens and opportunists worldwide
- Many disease-causing characteristics due to unique properties of their cell-wall
- Cell wall, slow replication time, and intra-cellular niche make treatment difficult and prolonged
- Immune response to infection responsible for large part of disease/pathology
- HIV infection strongly associated with *M.tb* and *M.avium* complex disease

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The Journal of Medicinal Chemistry 2012.51:1-16
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[www.TuberculosisTextbook.com](http://www.TuberculosisTextbook.com)