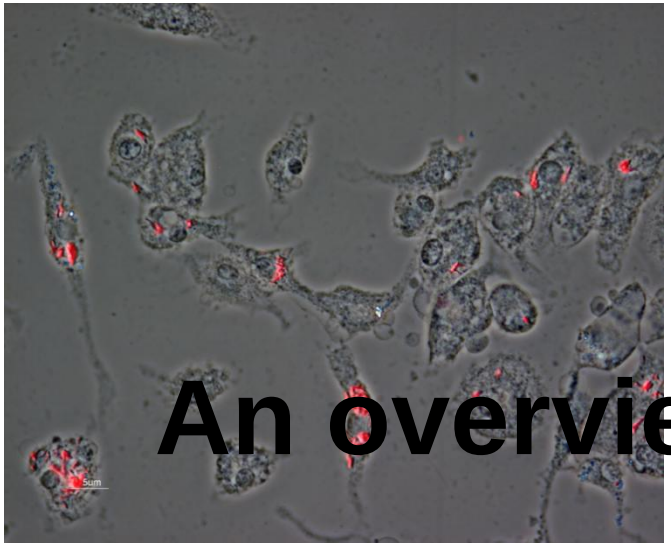
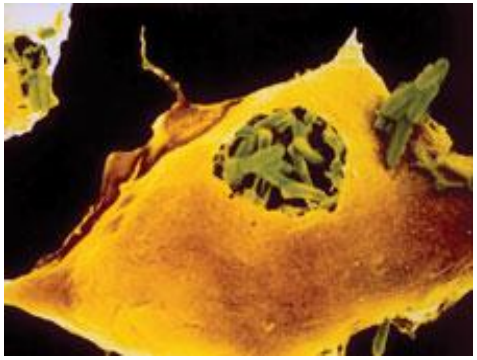


An overview of mycobacteria

Dr. Nitya Krishnan
CMMI, South Kensington campus



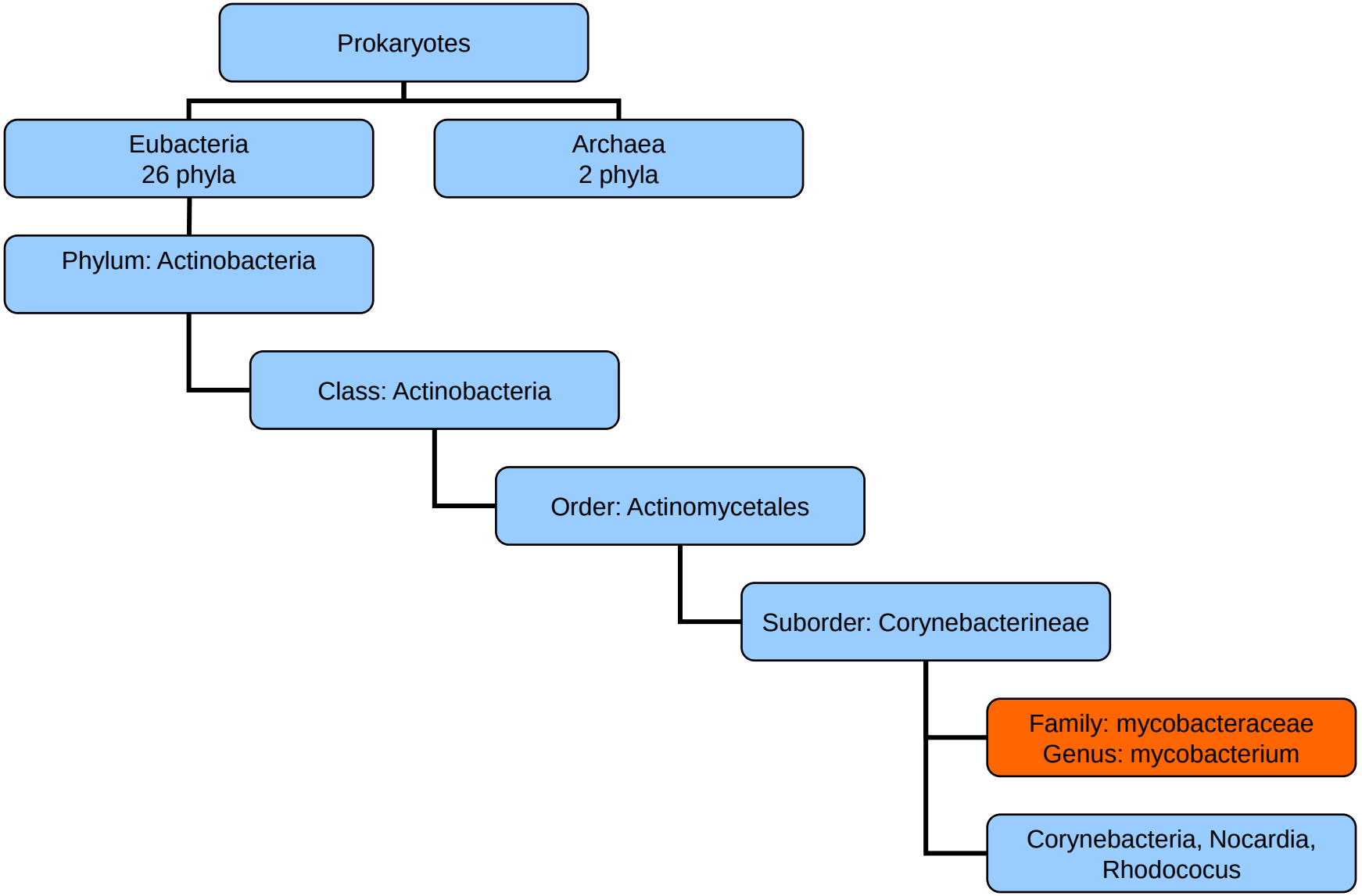
Learning outcomes

- 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

Classification



Medically important species

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

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

Mycobacteria and human disease

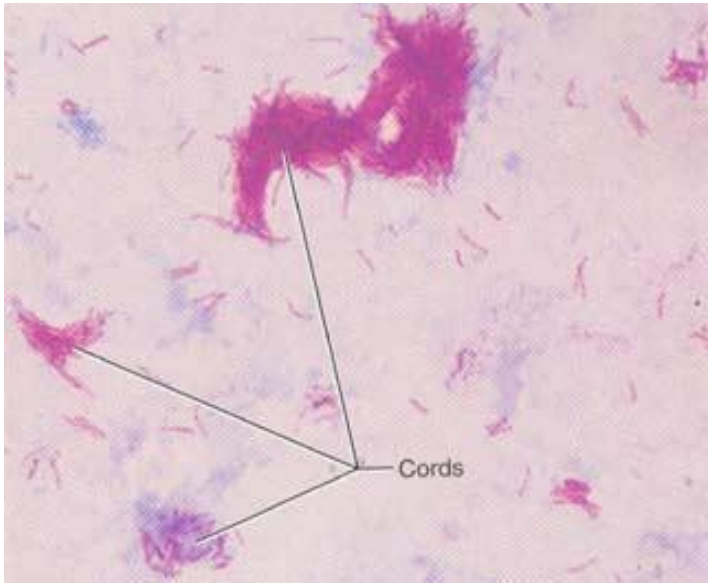
Disease in humans	**Mycobacterial species**
Tuberculosis	*M.tuberculosis* complex
Leprosy	*M.leprae*
Buruli ulcer	*M.ulcerans*
Swimming-pool granuloma	*M.marinum*
Post-traumatic abscesses	*M.fortuitum/M.chelonae*
Lymphadenitis (usually children)	*M.avium* complex
Opportunistic lung disease	*M.avium*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

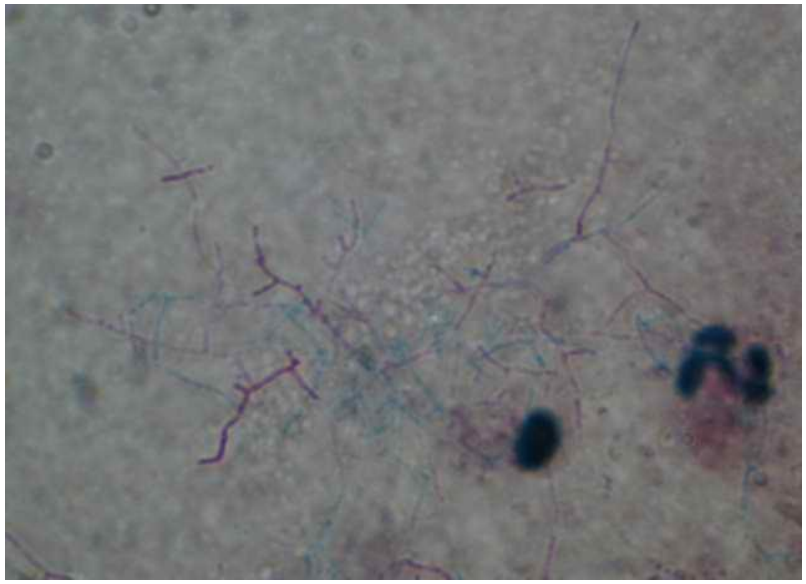
Defining ‘Clinical’ laboratory features of mycobacteria

- 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.gordonae)* and non-chromogens (*M.tuberculosis*)

What does ‘Acid-fast’ mean ?

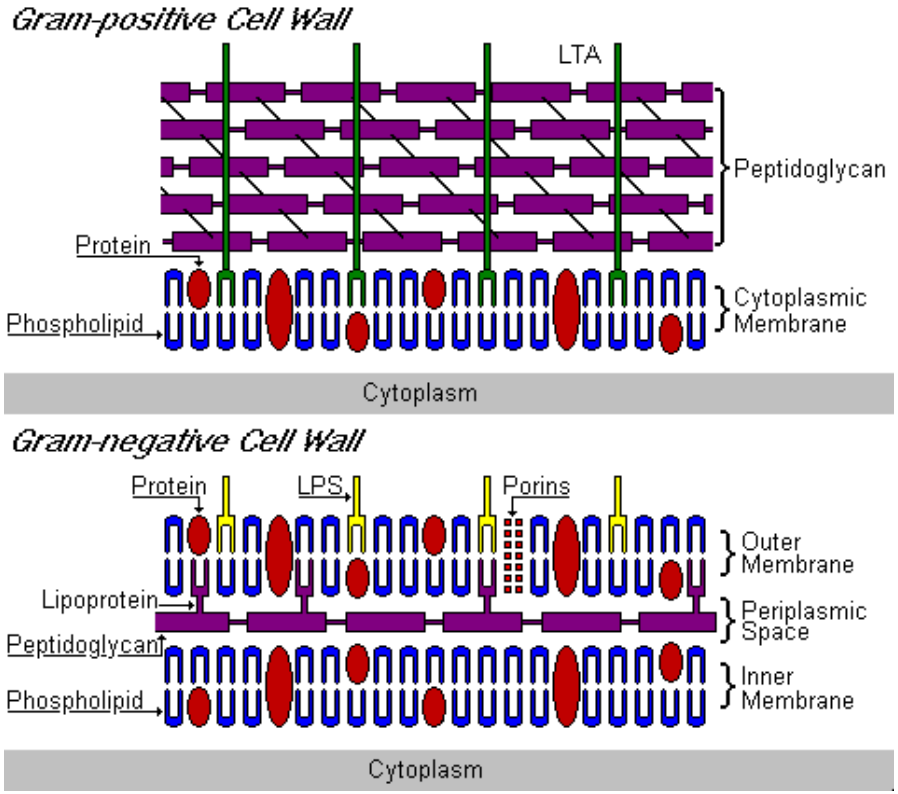
- Property discovered by Paul Ehrlich in 1883
- Adapted by Ziehl and Nielsen: the ‘ZN’ stain (1884)
- Definitive characteristic of all mycobacteria
- Other genus can be variably ‘acid-fast’: *nocardia* (branching)
- Ability to resist decolourisation with acidified alcohol when stained with an arylmethane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

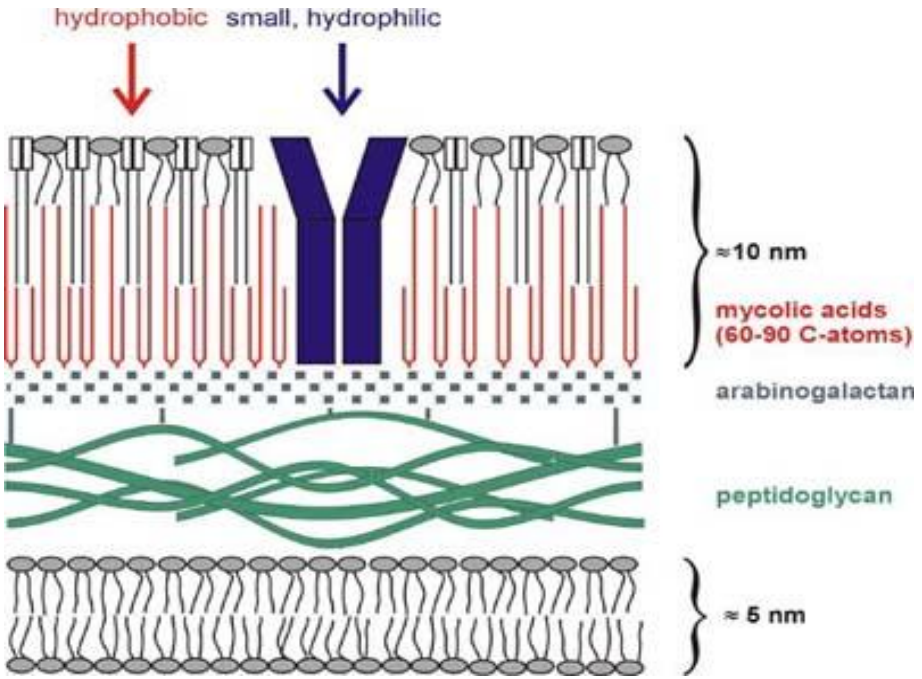




Why are mycobacteria acid fast?

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

rapid growing

M. smegmatis

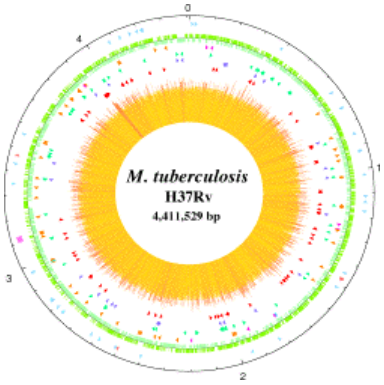
M. fortuitum

M. tuberculosis complex

M. avium-intracellulare

M. leprae

slow growing



Genome size

M. smegmatis	~7 Mb
M. tuberculosis	4.41 Mb
M. leprae	3.27 Mb

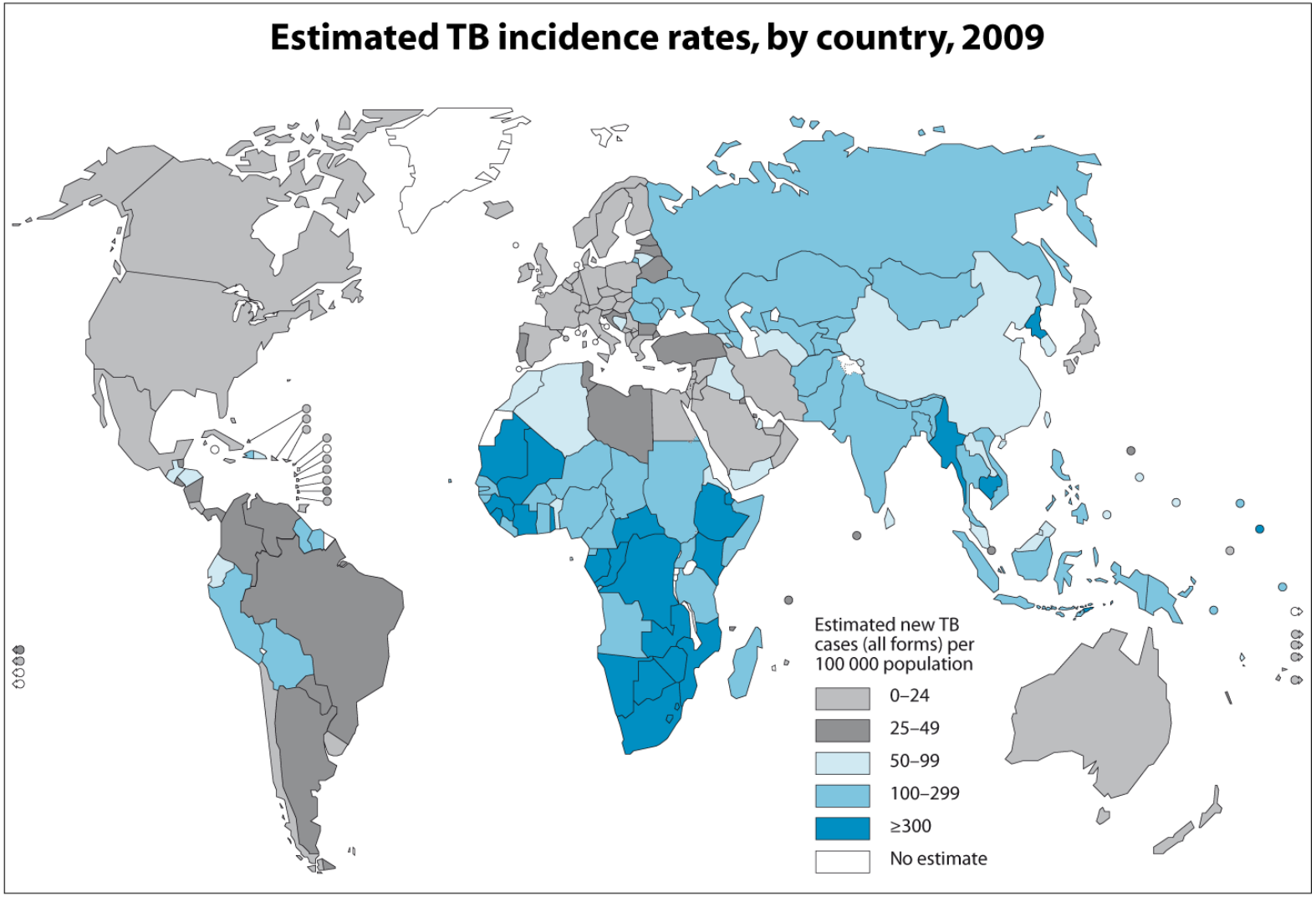
M. tuberculosis genome. Cole *et al*. 1998. Nature 393, 537-544

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



- 1/3rd 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

Illustration credit- WHO

Timeline - TB

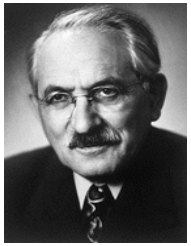
1882

Robert Koch

1921

BCG

1943



Selman Waksman

1960s

Current TB treatment course is developed

1981

1993

WHO declares TB a global emergency

1998

M.tb genome is sequenced

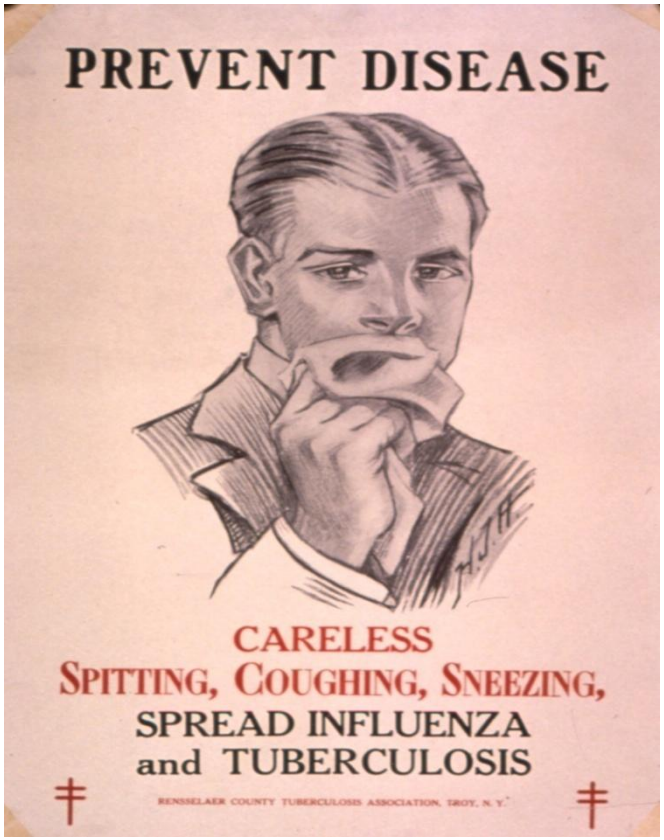
2006

Outbreak of X-DR TB

Tuberculosis: the disease

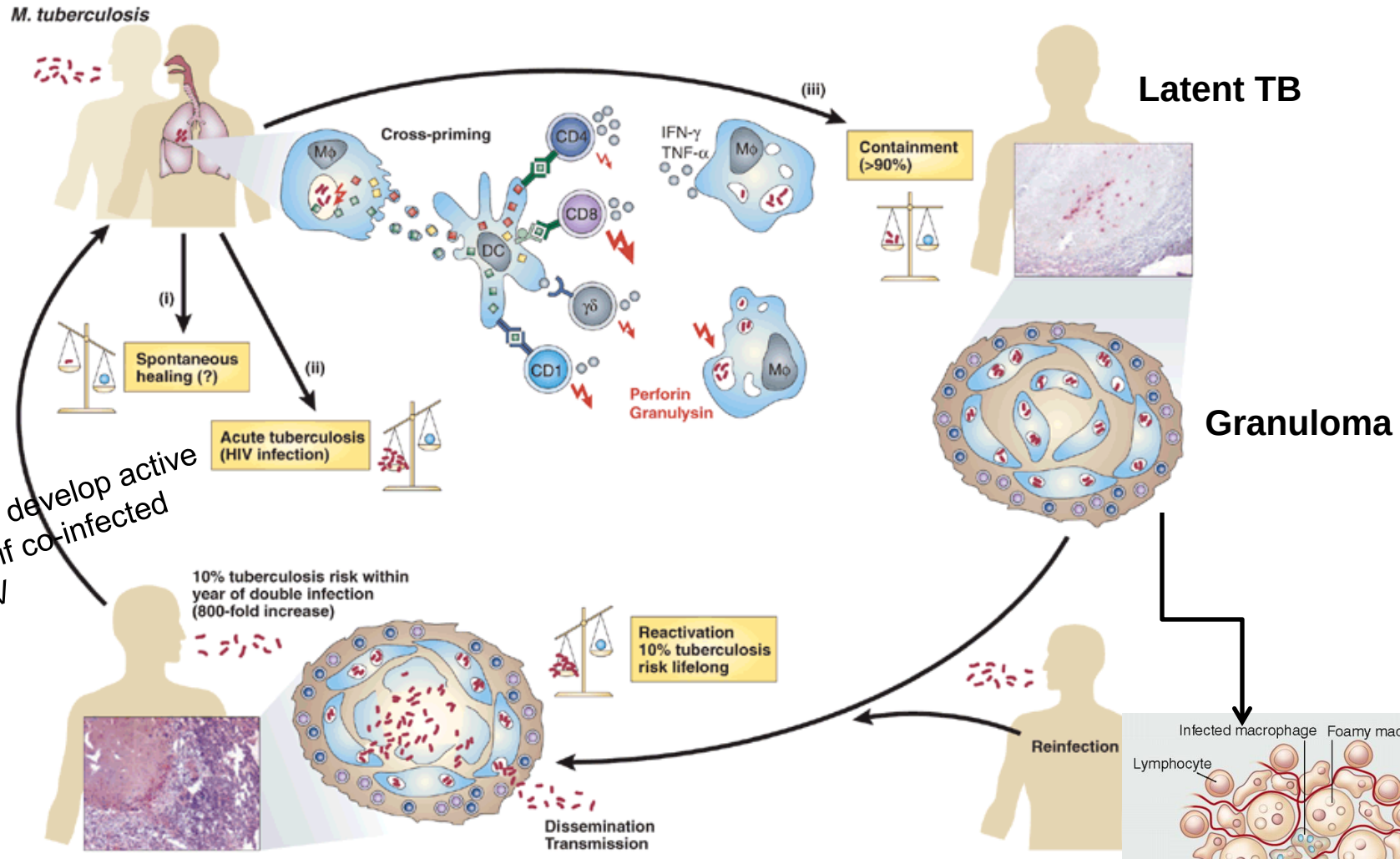
- 80% lung disease
- 20% extra-pulmonary disease
  - Lymph node
  - Brain
  - Bone
  - Kidney

Transmission of *M.tb*



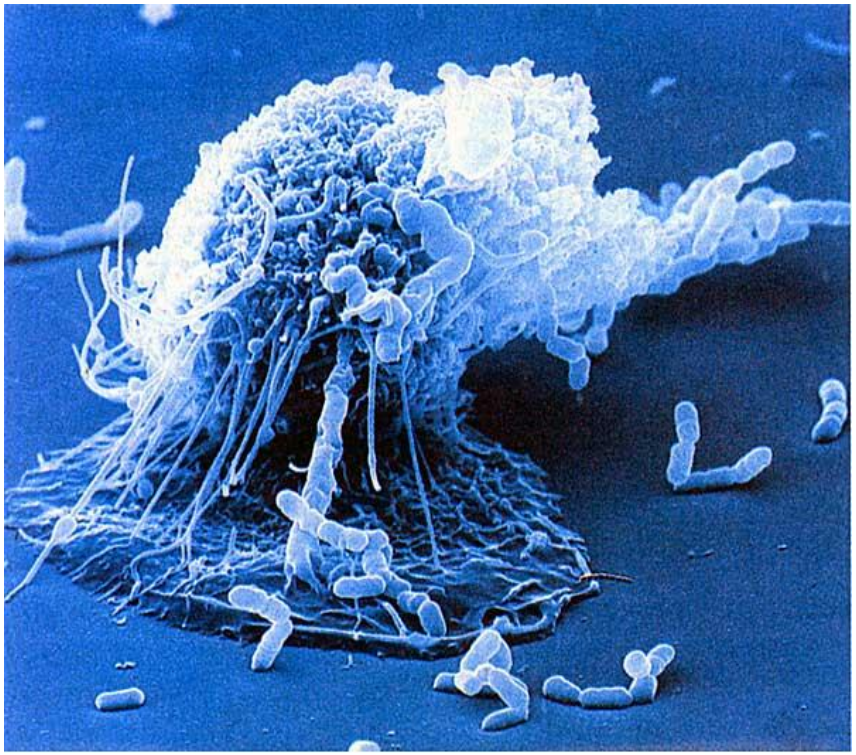
Rensselaer County Tuberculosis Association in Troy, NY. Poster dated- 1925

Life cycle of *M.tb*



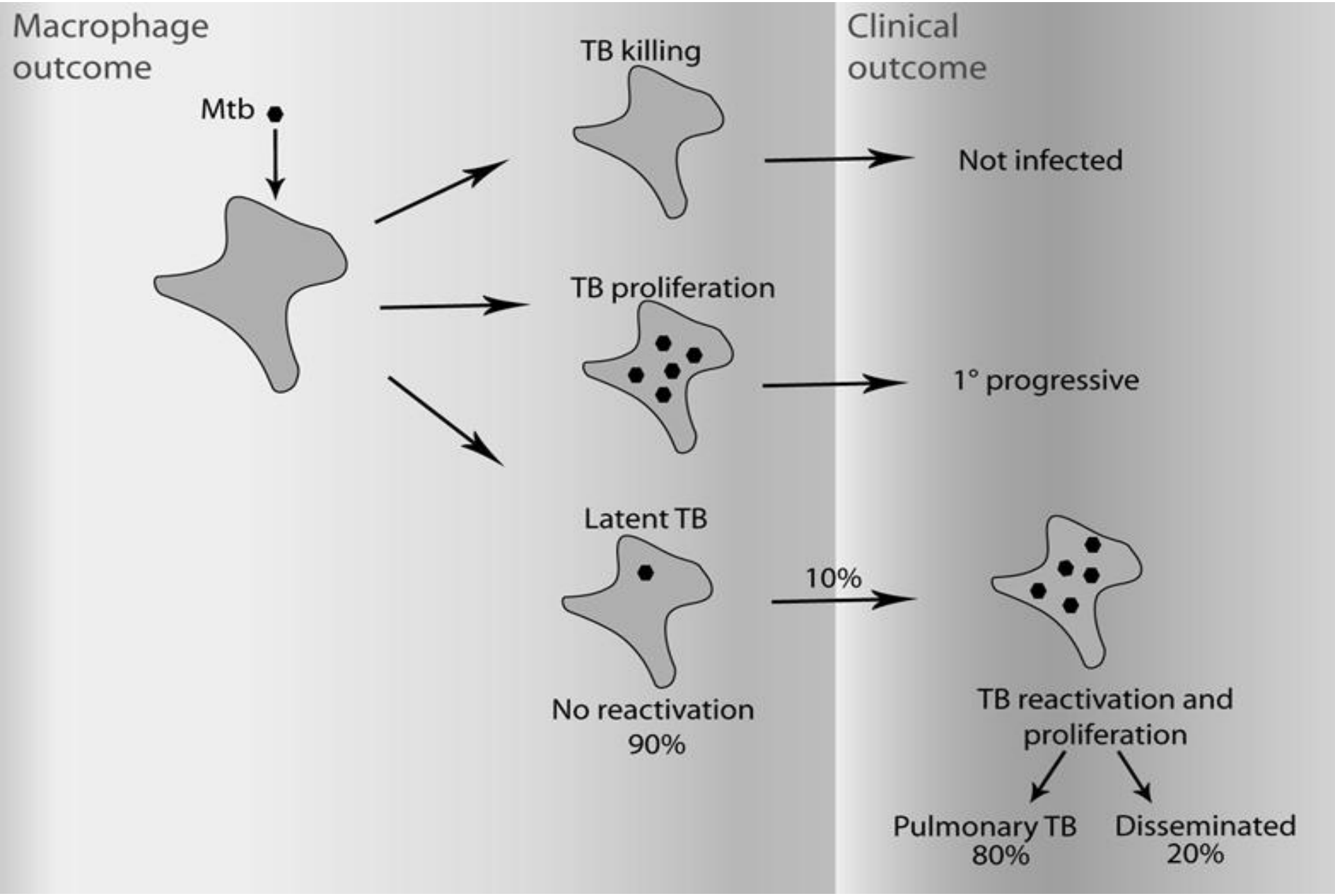
Nat Med, 2005,11:S33-S44

Host-pathogen interaction

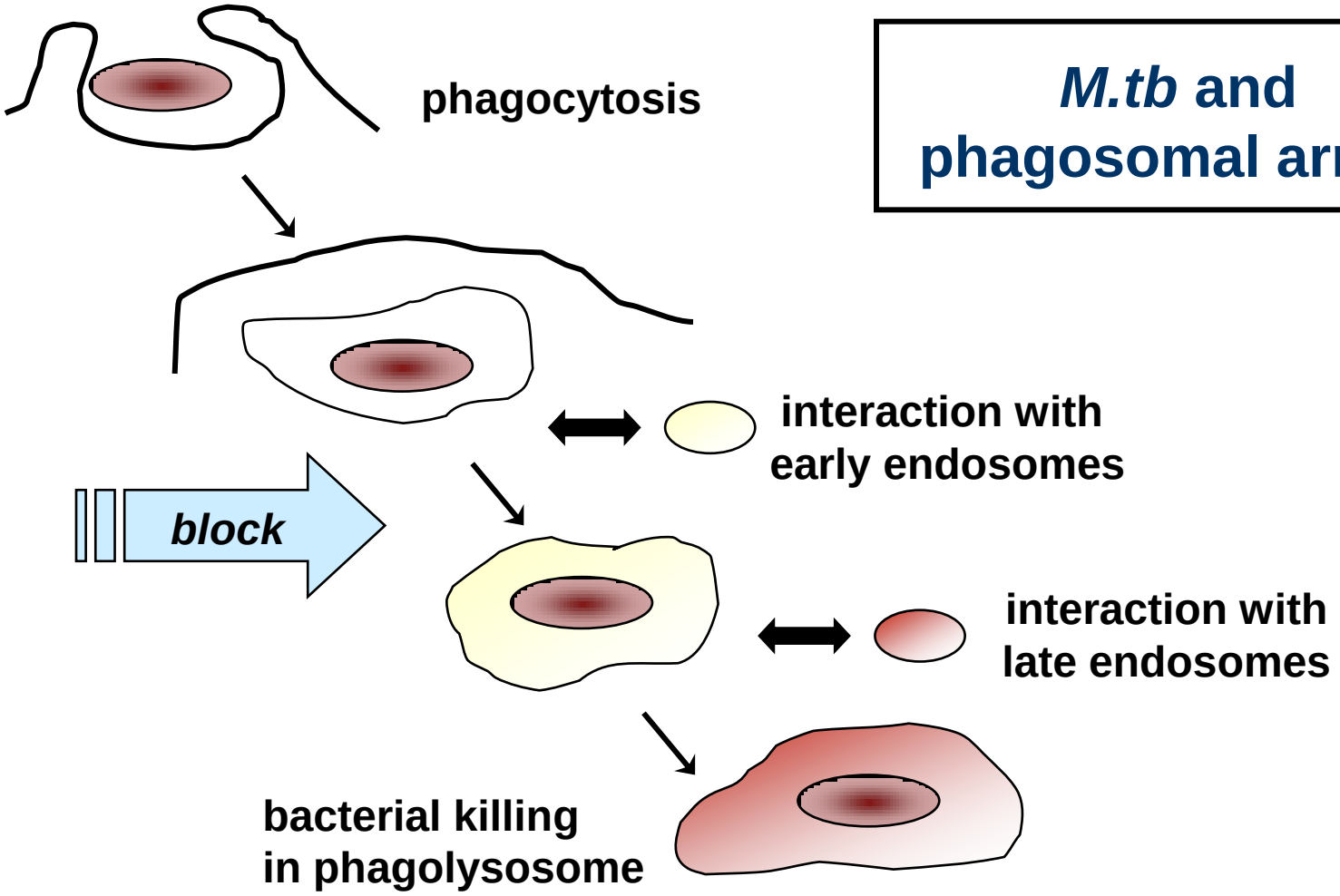


***M.tb* and macrophages**

M.tb and macrophages



M.tb and phagosomal arrest



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

T-cell mediated immunity

T-cells

- CD4
- CD8
- CD1
- γδ

IFN-γ
TNF-α

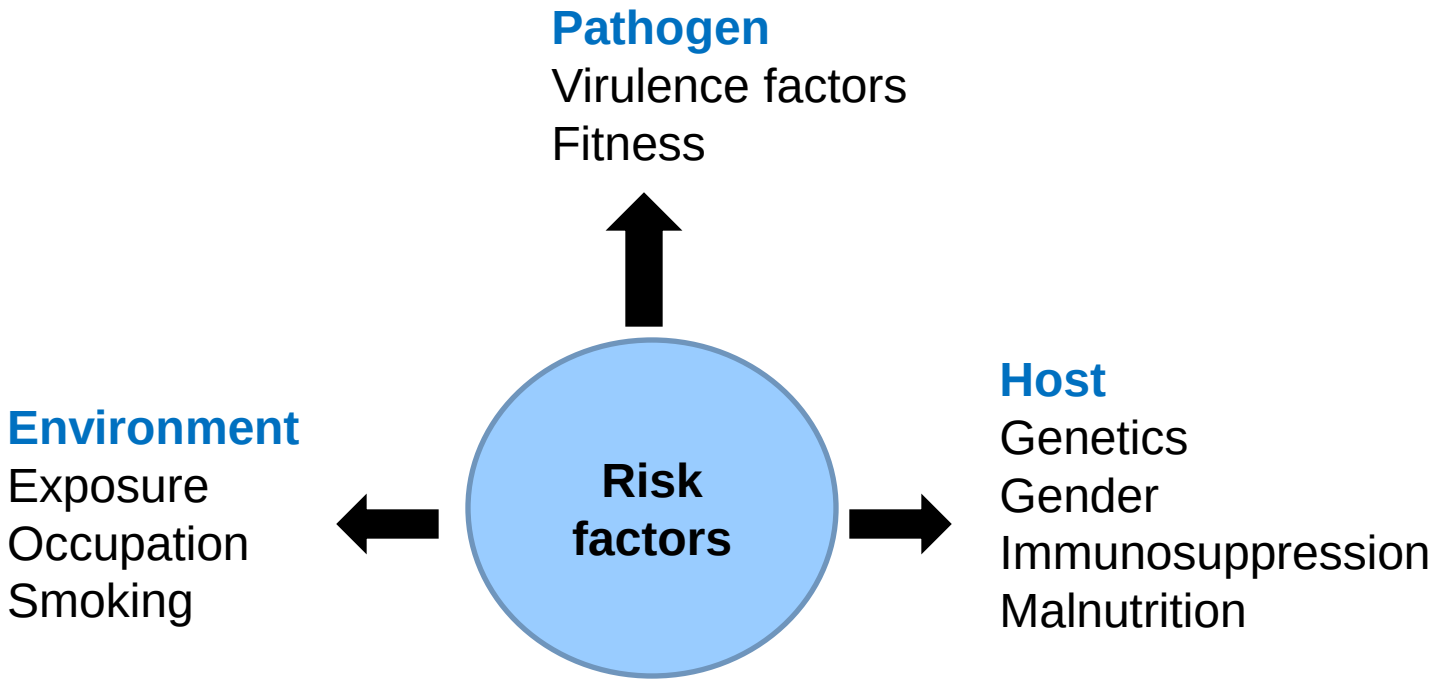
Control of infection

Infected macrophage

Perforin & Granulysin

HIV infection → ↓CD4 Tcells →

TB risk factors



Single gene defect- IFN-γ

- Maltese family
- Disseminated mycobacterial infection
- Failure to produce TNF-α and up-regulate IFN-γ
- Polymorphism in the IFN-γ 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

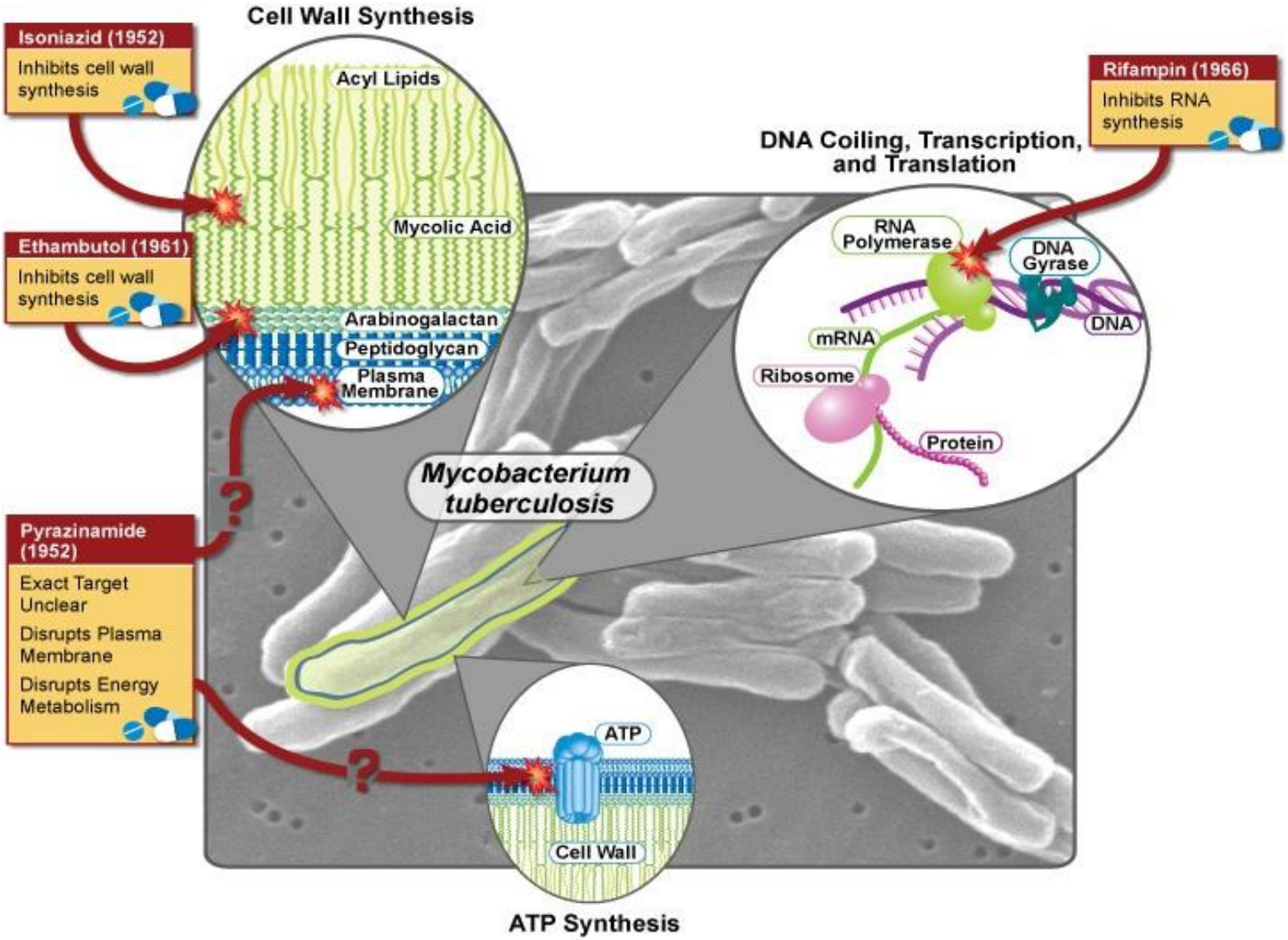
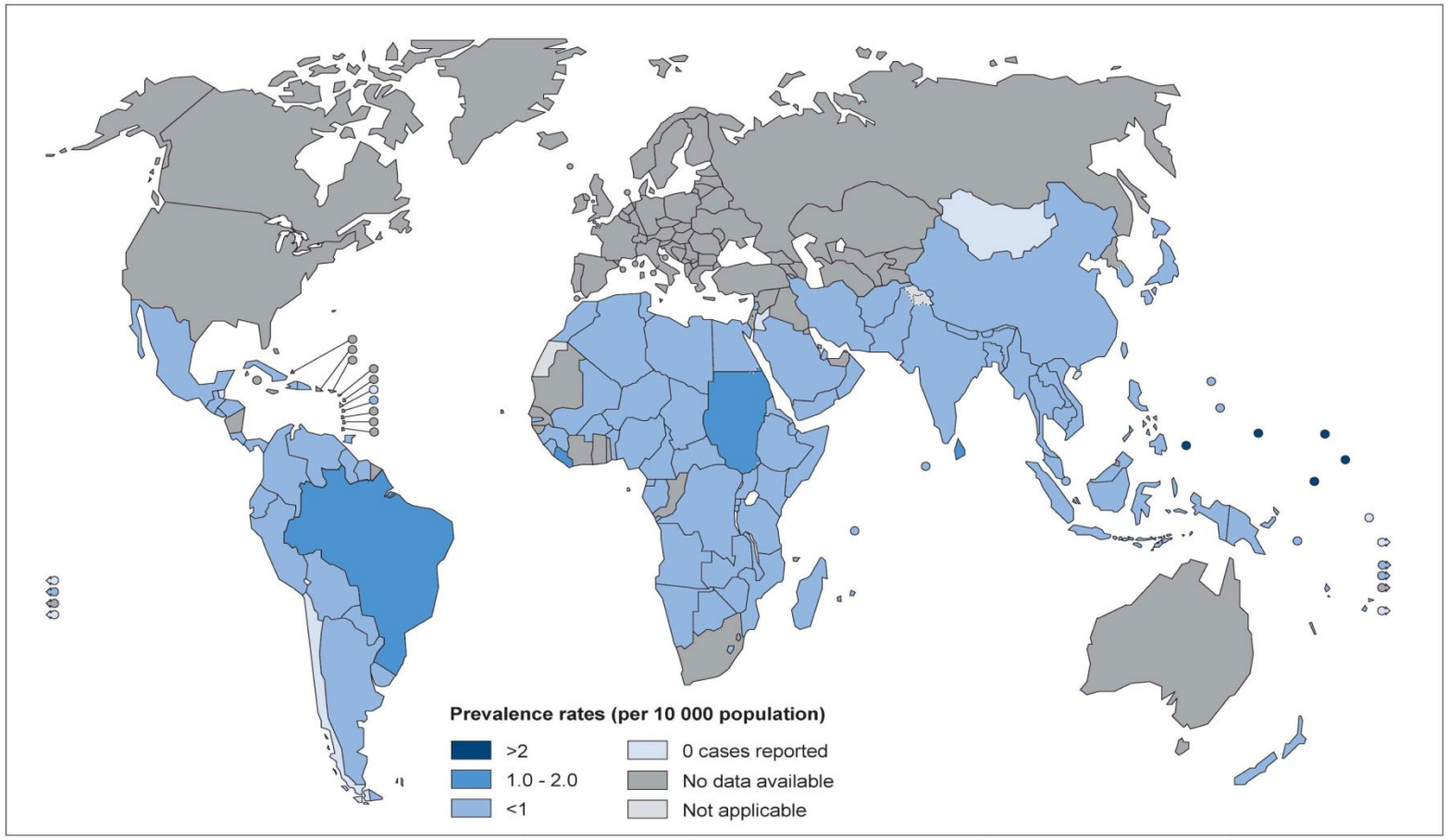


Illustration credit- NIAID

Leprosy and *M.leprae*

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

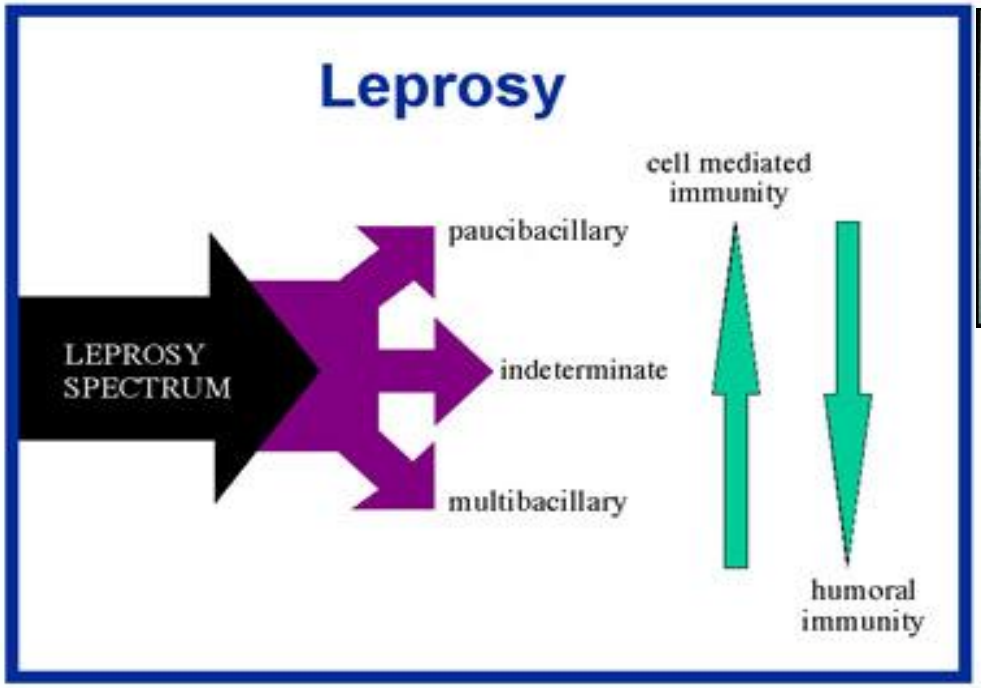
Leprosy prevalence rates, data reported to WHO as of beginning January 2011



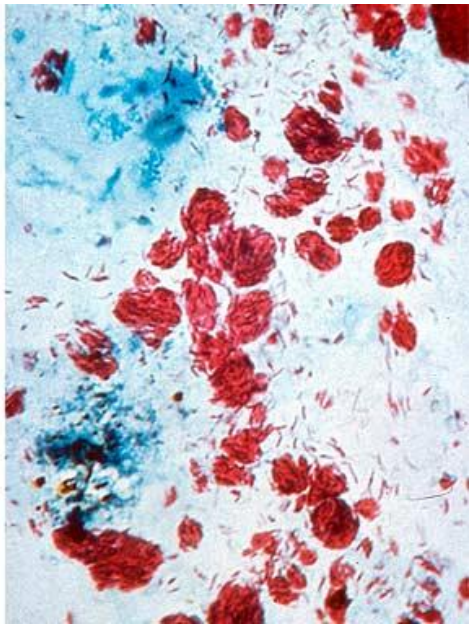
M.leprae

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

Leprosy


Leprosy
cell mediated immunity
paucibacillary
LEPROSY SPECTRUM
indeterminate
multibacillary
humoral immunity

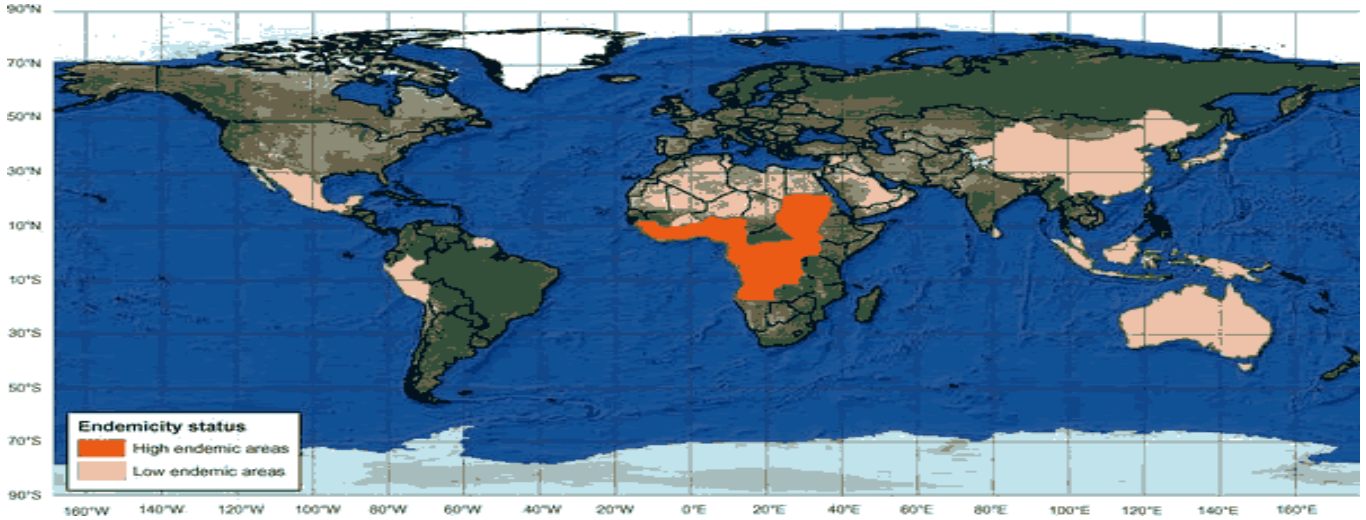
Leprosy: transmission and treatment



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

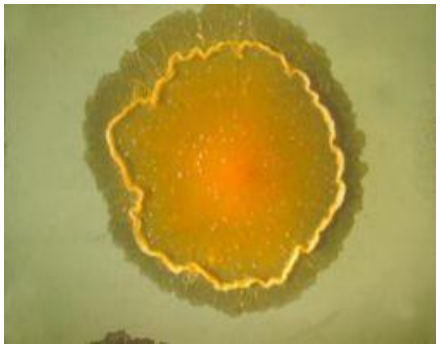
Buruli ulcer: *M.ulcerans*

- Environmental bacteria – may live within small aquatic organisms
- Transmission unknown
- Culturable at 27-30^{0}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

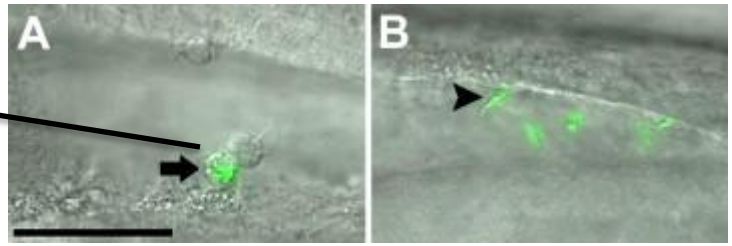
Swimming pool granuloma
M.marinum



- 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

References

•The secret lives of the pathogenic mycobacteria
Annual Reviews Microbiology 2003. 57:641–76

•Tuberculosis 2007: From basic science to patient care
www.TuberculosisTextbook.com

• Special section on Tuberculosis & Malaria
Science 2010.328:777-936

• Comparative pathogenesis of *Mycobacterium marinum* and *Mycobacterium tuberculosis*
Cellular Microbiology 2008.10:1027-1039

• T cells in mycobacterial infection and disease
Current Opinion in Immunology 2009.21:378-84