

Tuberculosis

*...Where youth grows pale, and spectre-thin,
and dies;...*

*You must not look at me in my dying gasp, nor
breathe my passing breath...*

*That drop of blood is my death warrant. I must
die.*

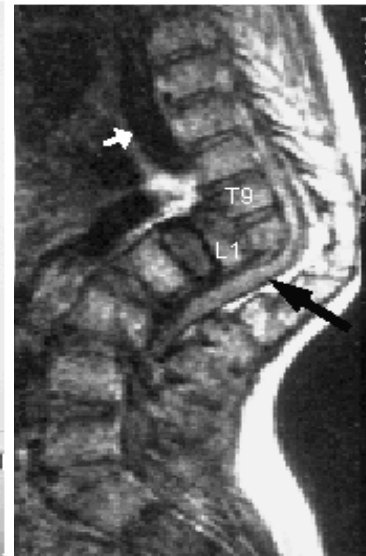
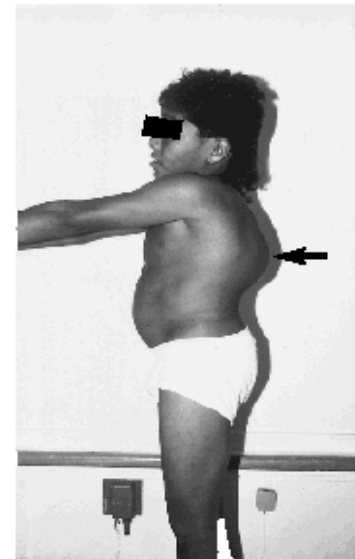
--John Keats (1795-1821)

History of TB

- Evidence found of TB in Egyptian mummies
- Appears in biblical scripture, Chinese lore (4000 BC), Indian religious books (2000 BC), and by the Greeks, Hippocrates and Aristotle (400 BC)
- Remains of mummified woman showed TB 500 years before Columbus arrived in the Americas

Variety of Names

- Great White Plague
 - Started in 1600s, continued for 200 years
- Phthisis (wasting)
- Scrofula (lymph node swelling)
- Pott's disease (spinal involvement)
- Consumption (*con*—completely; *sumere*—to take up)



Discovery

- 1720: Dr. Benjamin Marten conjectured that TB may have a causative agent, “wonderfully minute living creatures”
- 1882: Robert Koch discovered M. tuberculosis; disease killing 1 in 7 in Americas and Europe
- 1895: Wilhelm Konrad von Roentgen developed x-rays which helped with severity of disease

Spread and Containment

- Colonial period in America, TB affected rich and poor alike
- 1800: peak mortality 1600/100000
- 1840-1880: with industrialization, came spread from NE to MW to West
- 1886: Dr. Edward Trudeau opened first sanatorium, Saranac Lake, New York
- 1892: Dr. Herman Biggs started mandatory reporting of TB
- 1900: 10% of all deaths in US due to TB

Today

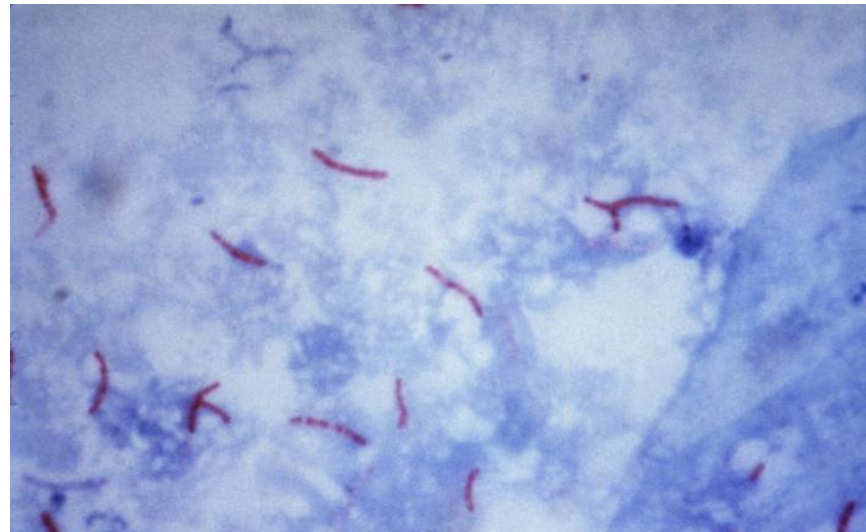
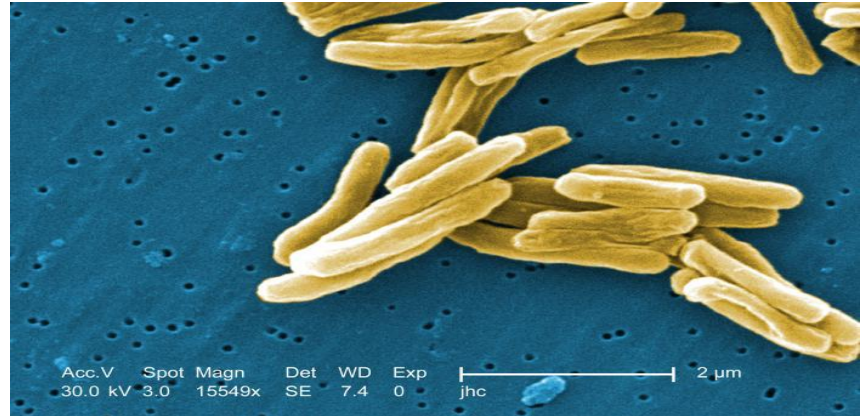
- 1/3 of world population infected with TB
 - World incidence, 2007, 1.39/100,000
- US rate lowest since recording of national rates
 - US rate, 2007, 4.2/100,000
- Texas and Houston/Harris County remain higher than US rate
 - Texas, 2007, 6.3/100,000
 - Houston area, 2007, 8.7/100,000

TB Epidemic

- Spike of TB 1986-1992
 - PH lost focus
 - HIV/AIDS
- Disease of disadvantaged
 - Institutionalized
 - Homeless, Drug Users
 - Alcoholism, Poor Nutrition
 - Immunocompromised, HIV
 - Foreign-born

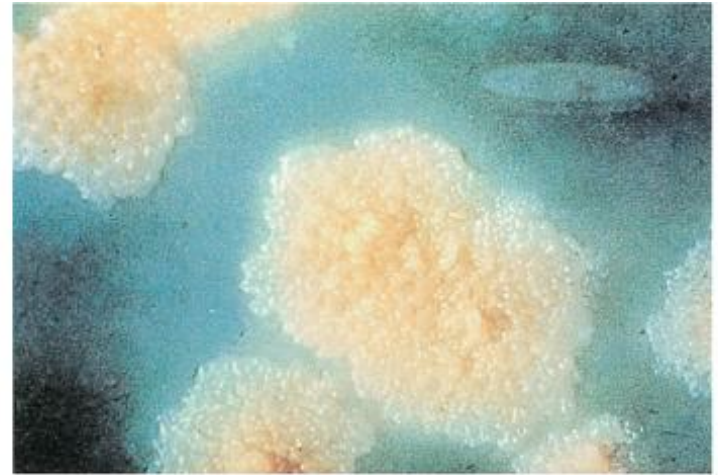
M. tuberculosis

- **Non-motile rods**
- **Obligate aerobe**
- **Intracellular parasite**
- **Acid-fast bacteria**
 - Impermeable to certain dyes/stains
 - Ziehl-Neelsen stain
 - Kinyoun's stain
- **Slow grower, divides every 18-24 hours**
- **Resistant to drying and chemical disinfectants**
- **Sensitive to heat (pasteurization) and UV light**



Are you there?

- **Middlebrook's medium:** agar based medium
- **Lowenstein-Jensen medium:** egg based medium
- MTB colonies small and buff colored on either medium
- Both types of media contain inhibitors to keep contaminants from out-growing MT
- Takes 4-6 weeks to get visual colonies on either type of media.
- **BACTEC system:** radio-labeled CO₂ released in the presence of MTB, 9-16 days

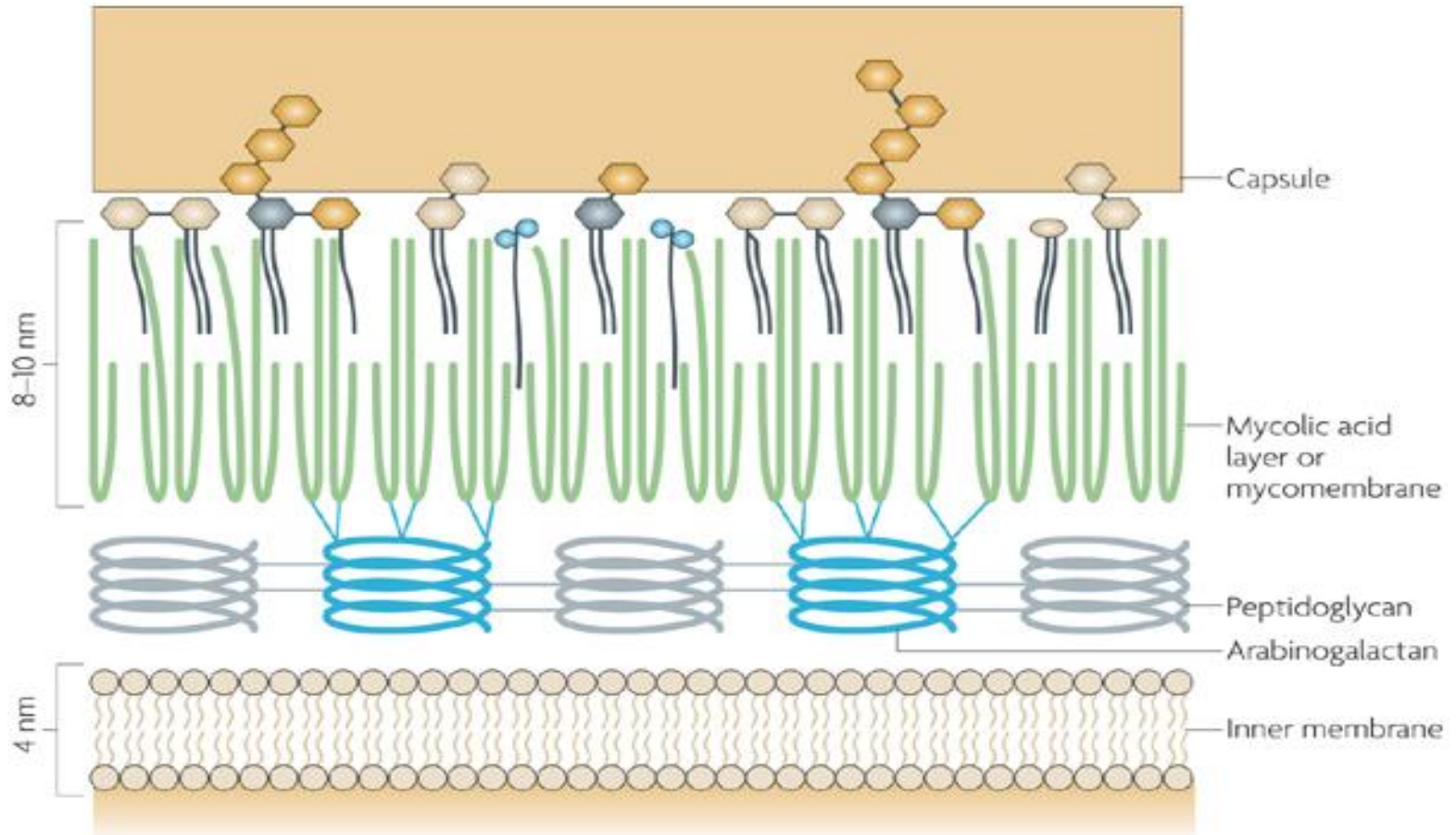


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MTB Cell Wall

- Peptidoglycan
- Complex lipids
 - 60% of cell wall
 - Impermeability to stains and dyes
 - Resistance to many antibiotics
 - Resistance to killing by acidic and alkaline compounds
 - Resistance to osmotic lysis via complement deposition
 - Resistance to lethal oxidations and survival inside of macrophages

MTB Cell Wall



Nature Reviews | Microbiology

www.nature.com/.../fig_tab/nrmicro1773_F1.html

Lipid structures in Cell Wall

- Mycolic acids
 - Hydrophobic molecules form lipid shell, affect permeability properties at cell surface
 - Likely prevent attack of cationic proteins, lysozyme, and oxygen radicals in the phagocytic granule.
 - Protect extracellular mycobacteria from complement deposition in serum.

Virulence Factors

- Cord factor
 - toxic to mammalian cells
 - inhibitor of PMN migration
 - most abundantly produced in virulent strains of MTB
- Wax-D
 - Major component of Freund's complete adjuvant (immunopotentiator of the immune system)

Virulence Mechanisms

- Not classic toxins, fimbriae, capsules
- Special mechanisms for cell entry
 - Binds directly to mannose receptors on macrophages via the cell wall-associated mannosylated glycolipid
 - LAM
 - Indirectly via certain complement receptors or Fc receptors
- Intracellular growth
 - Renders antibodies and complement ineffective
 - Inhibit phagosome-lysosome fusion in macrophages
- MTB interferes with the toxic effects of reactive oxygen intermediates

Infection versus Disease

- Infection (latent tuberculosis infection—LTBI):
 - MTB is in the body
 - Immune system keeping MTB in check by macrophages
 - No clinical symptoms/normal CXR
 - Positive TST or IGRA
- Disease (active tuberculosis disease/case):
 - MTB in body
 - Symptoms appear: weight loss, fatigue, hemoptysis, cough, fever
 - CXR abnormal; lesions present
 - Positive PPD or IGRA

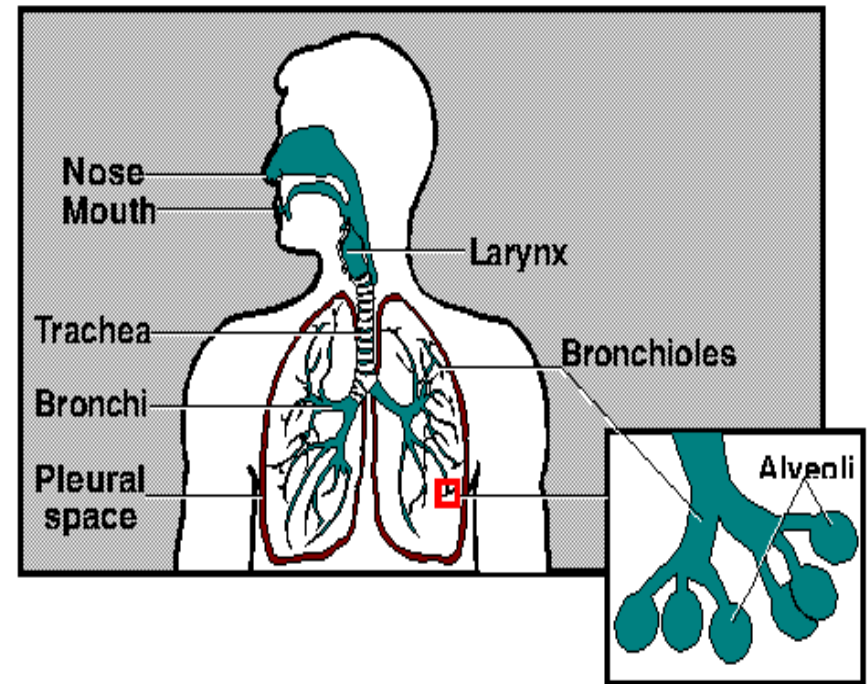
Chances

- Untreated LTBI with intact immunity
 - risk of developing symptomatic tuberculosis disease is 5% to 10% over lifetime
 - About half of that risk occurring during the first year or two after infection.
 - HIV co-infection increases to 5% to 10% per year

Exposure

Stage 1

- Coughing, singing, talking most effective for transmission
- Droplet nuclei containing MTB are inhaled into alveoli



Let's set up shop!

Stage 2

- 7-21 days after initial infection, MTB multiplies within un-activated macrophages until the macrophages burst
- Extravasation of macrophages begins from peripheral blood
- These cells are also un-activated and phagocytize MTB, but can not destroy the bacteria

Infiltration

Stage 3

- Lymphocyte infiltration—T cells
 - Recognize MTB antigen as foreign
 - T cells release IFN γ , activate macrophages
- CMI
 - Controls MTB infection
 - Results in unwanted pathology
- TST positive
- Tubercle formation/Caseating necrosis
 - MTB contained, but can remain dormant

Tuberculosis Immunity

- Cell-mediated immunity is protective

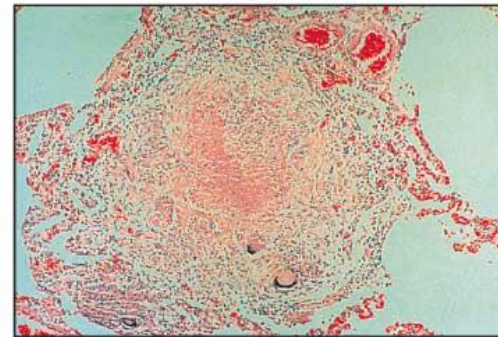
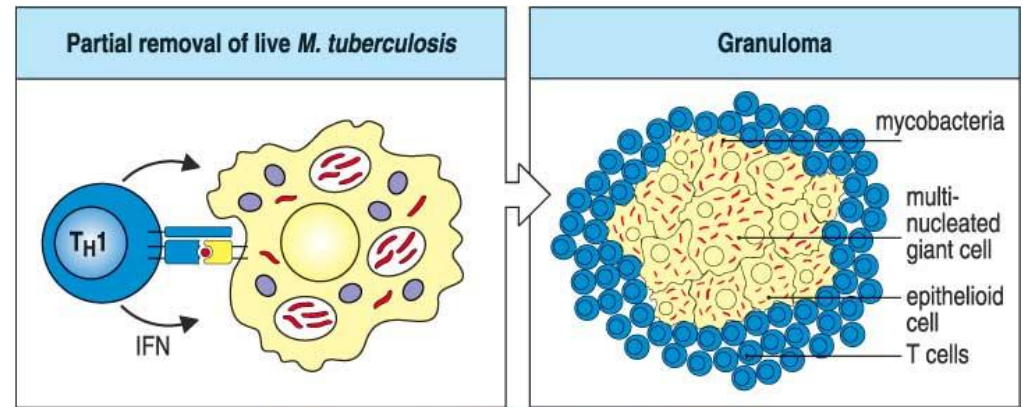
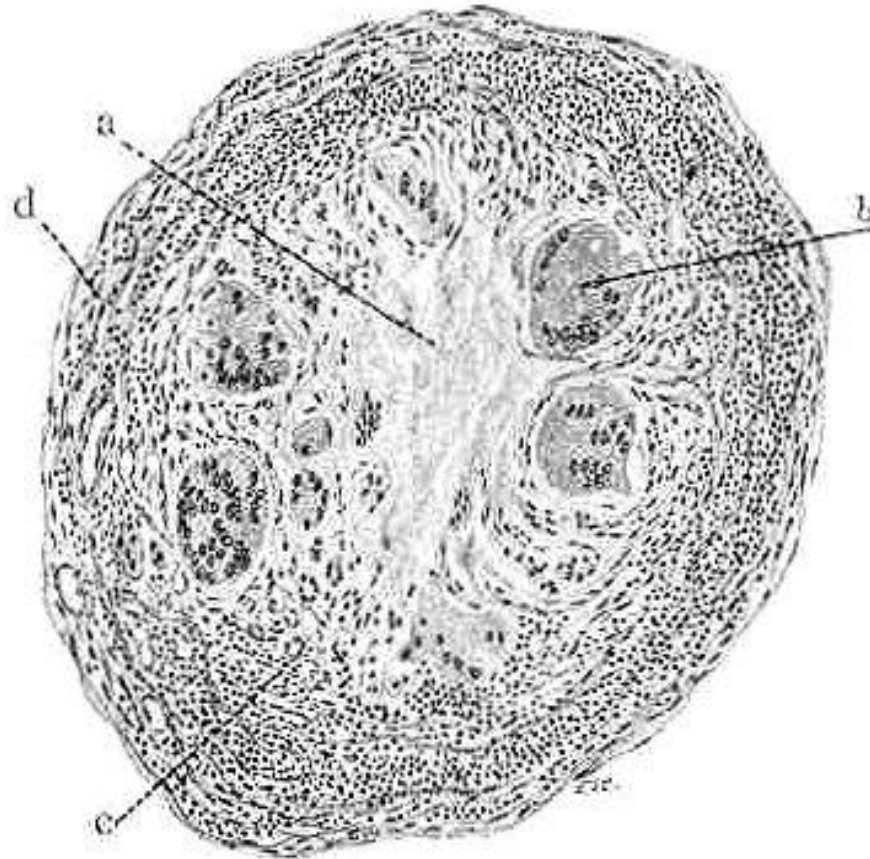


Fig 8.43 © 2001 Garland Science

TB Granuloma

FIG. 35



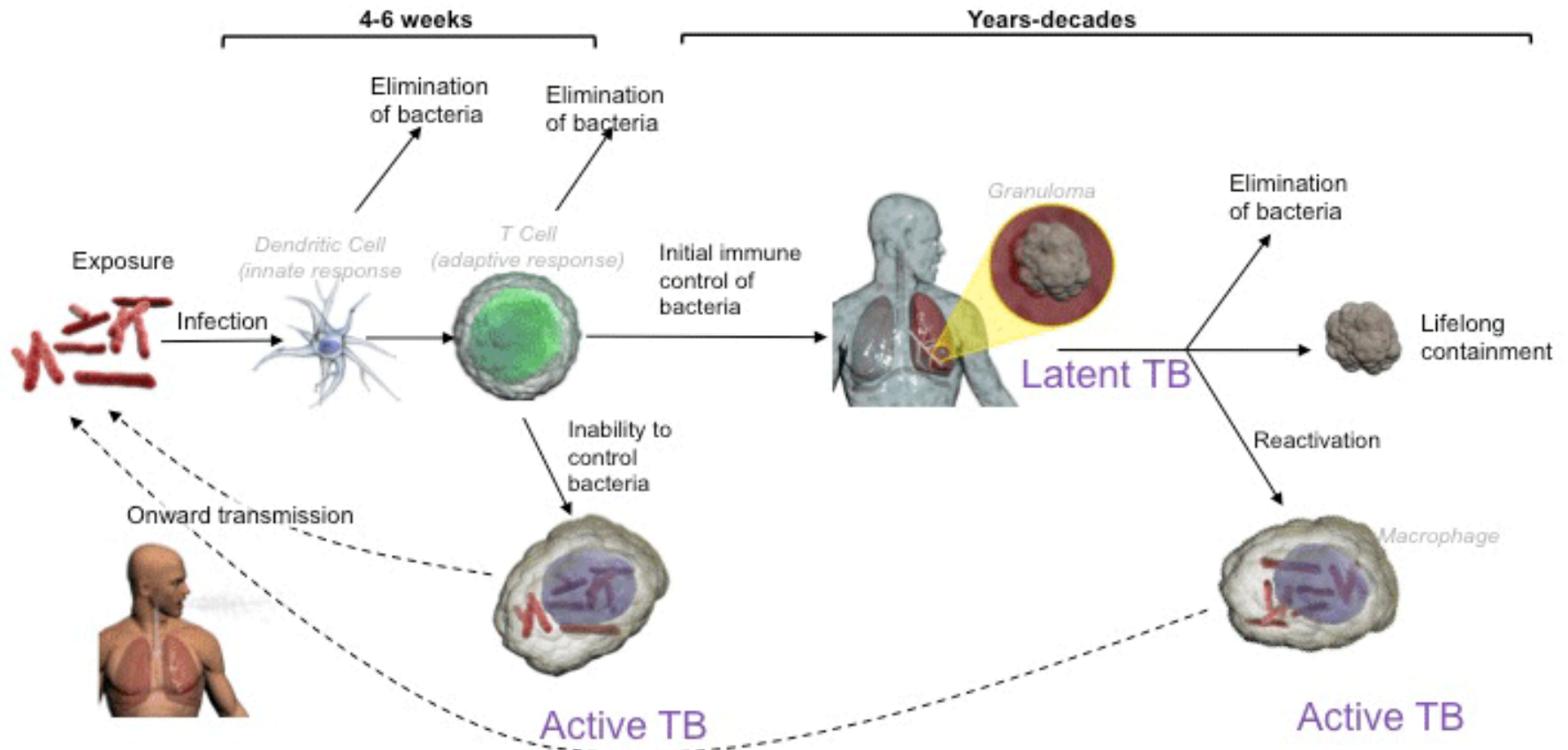
Tubercle from a case of tuberculosis of medium severity of the lung: *a*, central caseation; *b*, a giant cell; *c*, endothelial cells; *d*, connective-tissue zone infiltrated with lymphocytes.

Total invasion and Disease

Stage 4 and Stage 5

- Poor control by immune system
- Tubercle grows, may invade bronchus, other parts of lung
- If invades artery, metastasizes to other parts, miliary TB
- Tubercle may liquefy, resulting in rapid multiplication, eventually bursting through wall
 - Forms cavity
 - Ghon complex

Natural history of TB infection



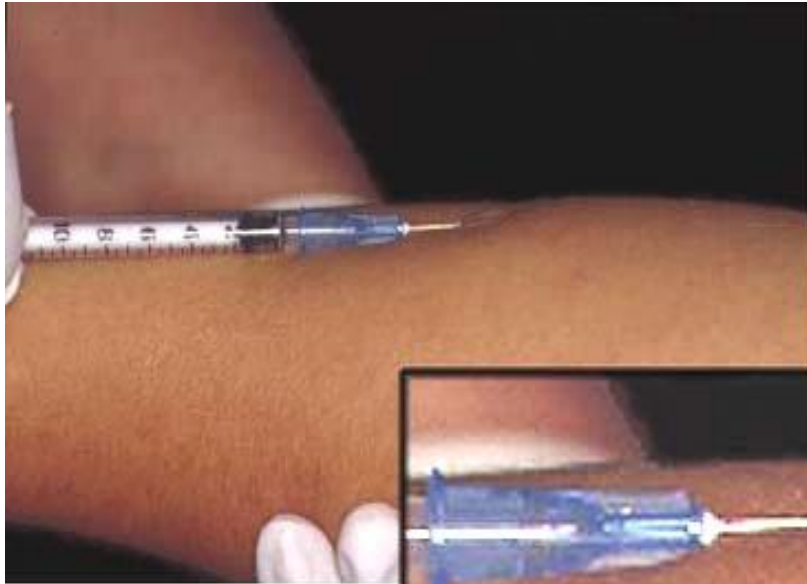
TB Diagnosis

- Exposure
- PPD/IGRA
- Clinical Signs and Symptoms
- Sputum smear and culture
- Chest Xray
- Diagnosis made from culture or clinically

TST, PPD, or Mantoux

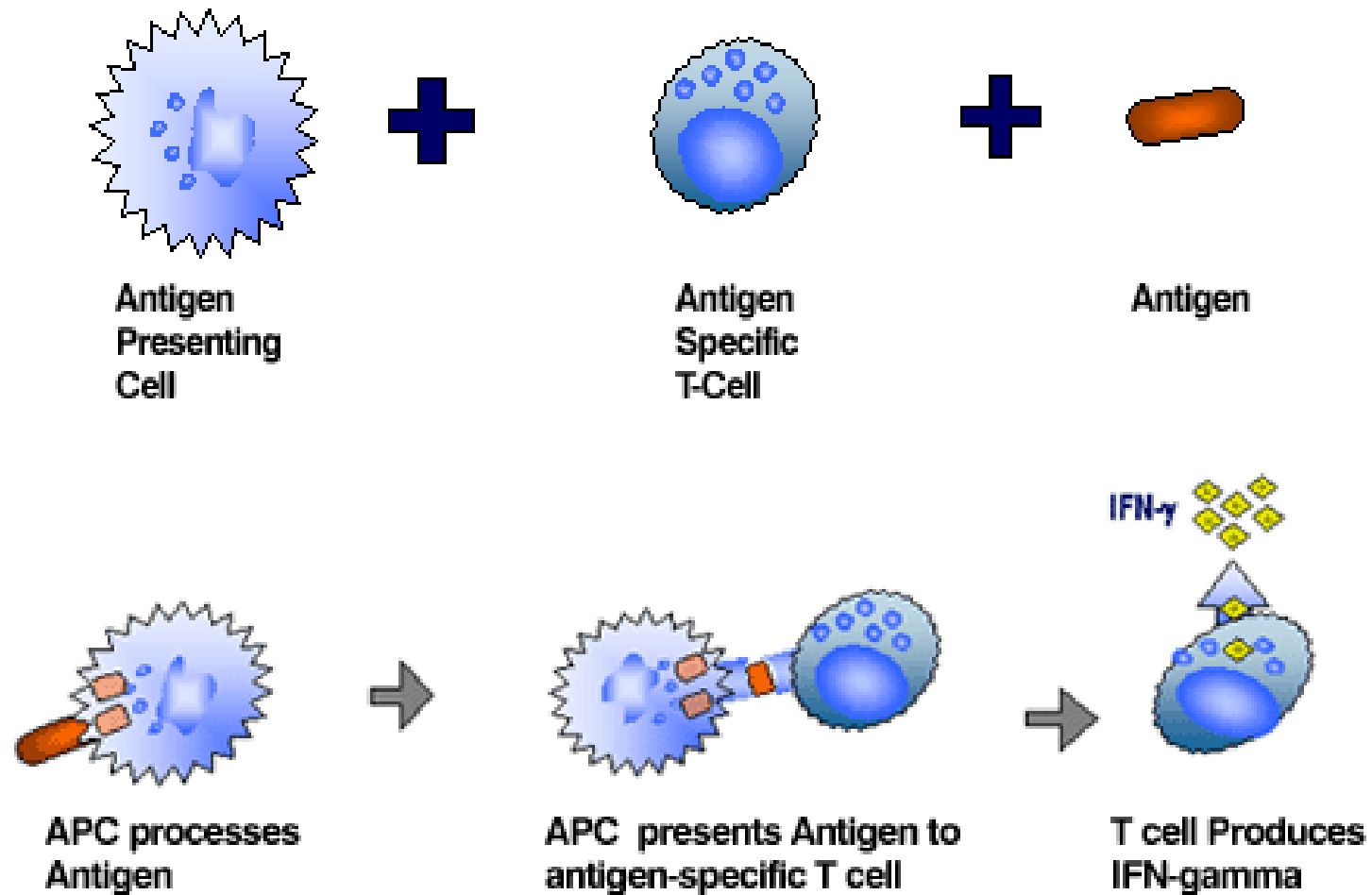
- Purified Protein Derivative (PPD): tuberculin from MTB organisms injected
 - Elicits delayed type hypersensitivity reaction at injection site
 - T cells migrate to skin site and release chemical messengers called lymphokines resulting in induration and redness
- Need new alternative
 - Immunocompromised may not respond, HIV
 - Cross-reacts with environmental mycobacteria and BCG, boosting
 - Takes 48-72 hours, compliance low
 - Subjective readings, and variable cutoff points (5-15 mm)

PPD



Interferon Gamma Release Assays

- Quantiferon-Gold (QFT-G)
 - Tests cell mediated immunity to simulated MTB peptide antigens, ESAT-6 and CFP-10
 - Small segment of MTB genome contains these specific antigens in the Region of Difference (RD1) 1 region
 - Lost through the passage of *M bovis* by Calmette and Guerin to produce the BCG vaccine
 - White blood cells separated and incubated with these two antigens
 - White blood cells release IFN-gamma if person infected, amount of IFN γ measured by ELISA



The stimulation of effector T cells in whole blood with a specific antigen(s) or mitogen, and the subsequent simple quantification of the resulting IFN- in the plasma, is the basis of the QuantiFERON technology.

Interferon Gamma Release Assays

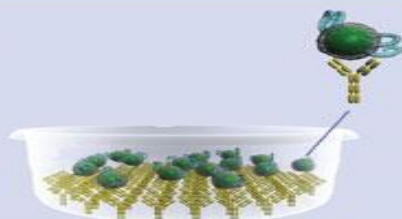
- TSPOT.TB
 - Wells of plates are coated with IFN- γ antibodies
 - PBMCs are incubated with ESAT-6 and CFP-10 to sensitize T cells in these wells
 - Activated effector T cells, both CD4+ and CD8+, produce IFN- γ and bind to IFN- γ antibodies
 - Enzyme-linked immunospot (ELISpot) methodology enumerate sensitized T cells that have released IFN- γ

1.



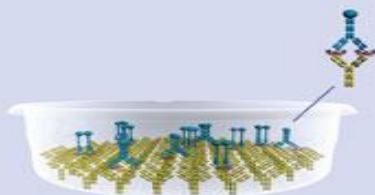
Collect the blood sample. At the lab, PBMCs are separated from whole blood, washed, counted and inoculated into 4 separate microtiter wells.

2.



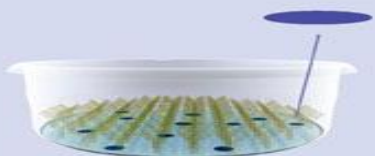
Add WBCs [●] and specific TB antigens [⊕] to wells pre-coated with antibodies to IFN- γ [Y] and incubate 16 to 20 hours (37° C, CO₂).

3.



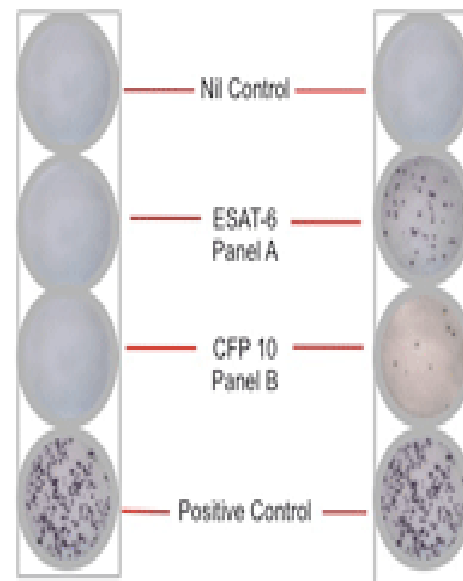
IFN- γ [⊕] is released from activated T cells and captured. Wash wells, add secondary conjugated antibody [Y]. Incubate for one hour.

4.



Wells are washed. A substrate is added which produces spots [■] where interferon gamma was secreted by T cells. Spots are counted.

Interpretation of Results



Negative
Result

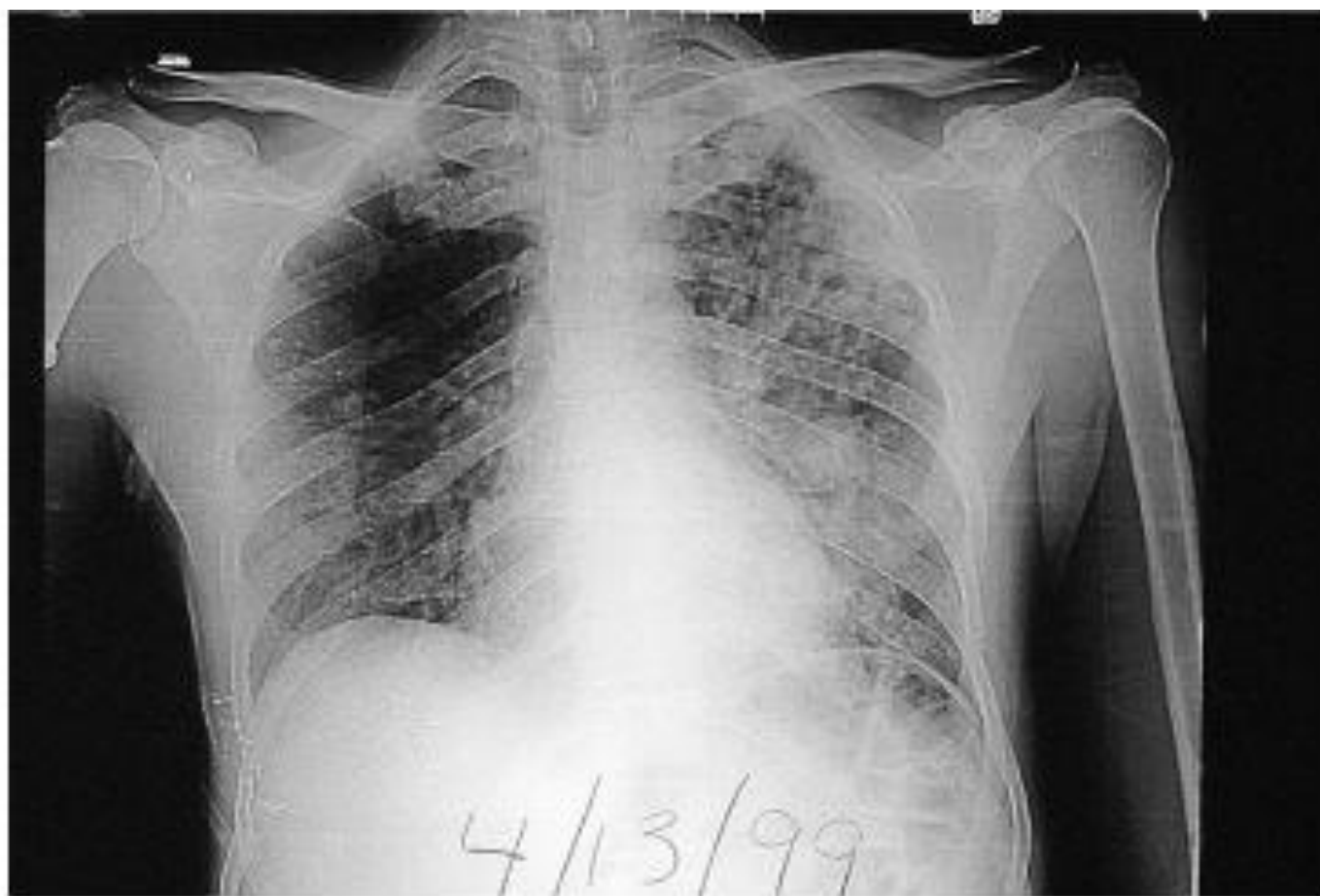
Positive
Result



Stop TB. Start with **T-SPOT.TB**

Why IGRAs

- Advantages
 - One time visit
 - No boosting or cross-reactivity
 - RD1 region not intact in non-MTB complex
 - Results in 24 hours
 - Objective results
- Disadvantages
 - Lab errors can occur
 - Process blood samples within 12 hr., GIT more time
 - Cost
 - Need more longitudinal studies



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BCG vaccine

- bacille Calmette-Guérin
- BCG recommended in foreign countries in infancy
- Prevents childhood tuberculous meningitis and miliary disease
- Not generally recommended in US
 - low risk of infection with *Mycobacterium tuberculosis*
 - variable effectiveness against adult pulmonary TB
 - vaccine's potential interference with TST reactivity
 - In US, consult with TB expert before BCG given
- Created from *Mycobacterium bovis*
 - Live attenuated strain of *M bovis*

Treatment of LTBI

- LTBI
 - Rule out active disease
 - Optimal to prevent disease activation
 - 6-9 months of isoniazid or 4 months of rifampin
 - Hepatic disease or disorders complicate therapy
 - Can refuse treatment at this stage

Treatment of TB Disease

- Reportable Disease/Contact Tracing
- DOT
- 4 drug regimen for up to 7 months
 - Isoniazid
 - Ethambutol
 - Rifampin (HIV pts use rifabutin)
 - Pyrazinamide
- MDR-TB/XDR-TB

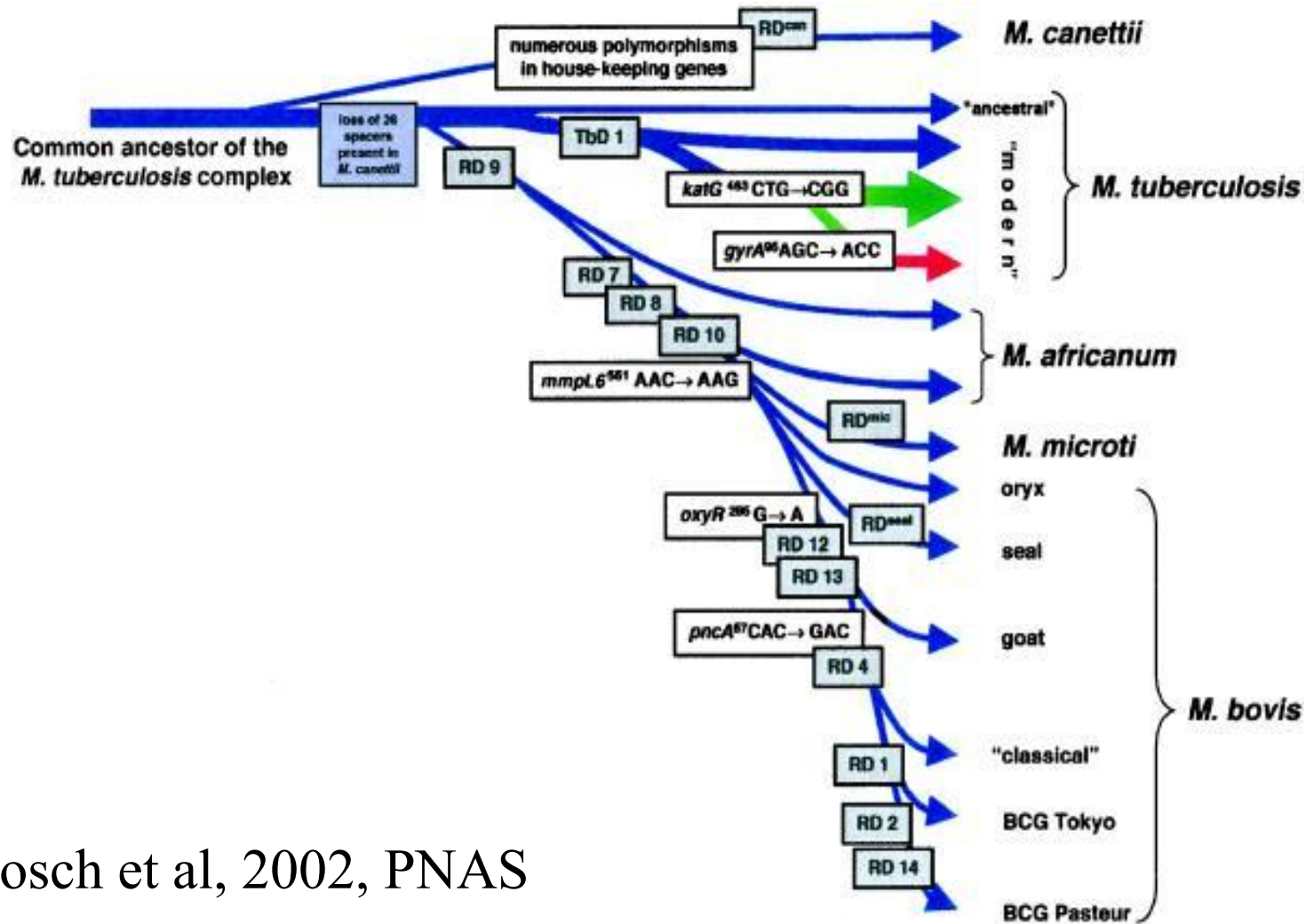
Mycobacterium tuberculosis complex

- Intracellular pathogens of animals and humans
- *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*
 - cause the disease known as tuberculosis (TB)
 - members of the tuberculosis species complex
 - *M. bovis* is usually pathogenic for animals
 - Extrapulmonary TB from contaminated milk)

Mycobacterium other than tuberculosis (MOTT)

- Environmental mycobacteria
 - *M kansasii*, *M marinum*, *M szulgai*
 - May cross react with QFT
- *Mycobacterium avium* complex
 - Immunosuppressed, especially HIV
- *Mycobacterium leprae*
 - leprosy

Evolution



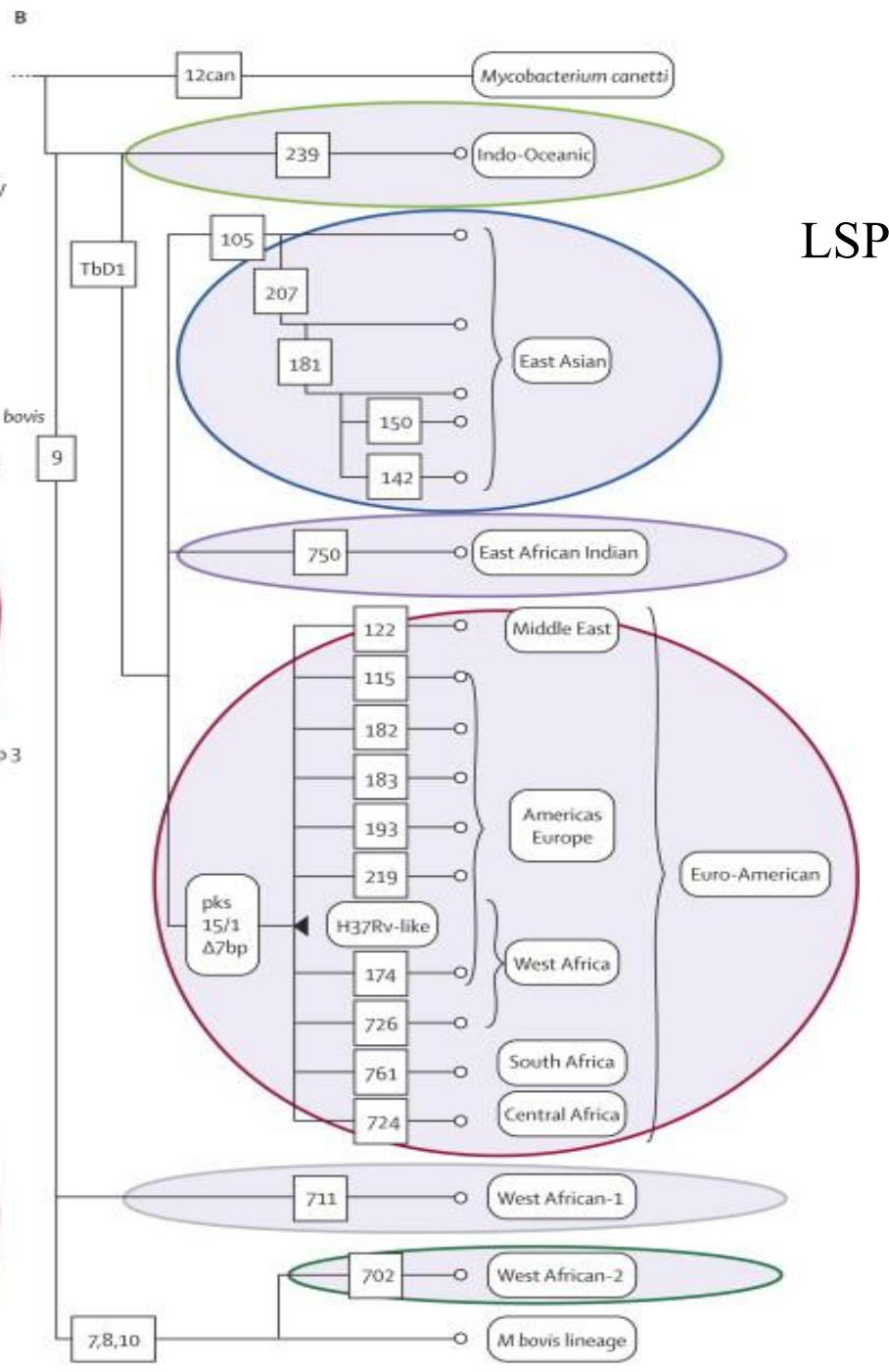
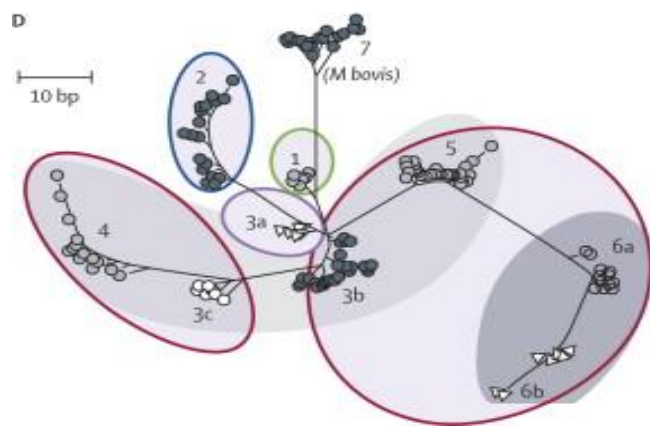
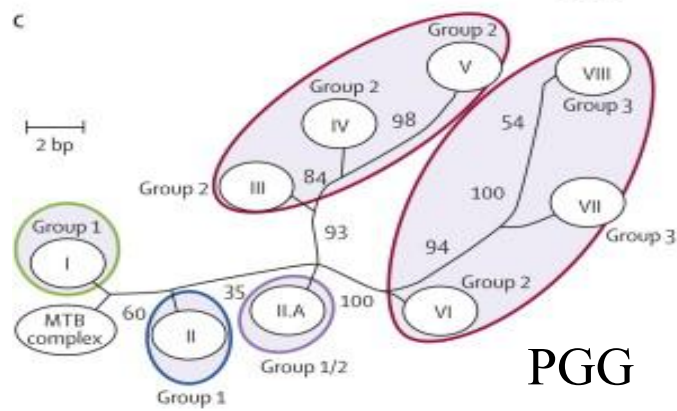
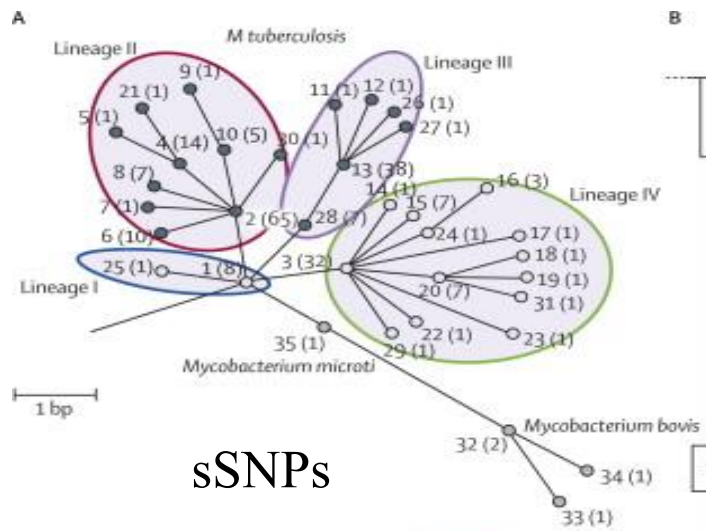
Brosch et al, 2002, PNAS

Summary

Brosch et al.

- MTB did not originate from *M bovis*
 - *Ancestral strains were all human pathogens*
- Ancestral strains endemic in Africa and India
- Modern strains (deleted TbD1 region) are responsible for worldwide epidemic

	Lineage 1	Lineage 2	Lineage 3	Lineage 4	Lineage 5	Lineage 6
SNP (Sreevatsan et al ⁴)	Principal genetic group 1	Principal genetic group 1	Principal genetic group 1	Principal genetic groups 2 and 3	Principal genetic group 1	Principal genetic group 1
SNP (Baker et al ⁹³)	Lineage IV	Lineage I	Lineage III	Lineage II	Not done	Not done
LSP (Gagneux et al ⁸⁶)	Indo-Oceanic lineage	East Asian lineage	East African-Indian lineage	Euro-American lineage	West African lineage I	West African lineage II
SNP (Gutacker et al ⁶⁷)	Cluster I	Cluster II	Cluster II.A	Clusters III-VII	Not done	Not done
SNP (Filliol et al ⁶⁰)	Cluster group 1	Cluster group 2	Cluster group 3a	Cluster groups 3b-6b	Not done	Not done
Spoligotyping (Brudey et al ⁶²)	EAI	Beijing	CAS	Haarlem, LAM, T, X	AFRI2	AFRI1
LSP marker ⁸⁶	RD239	RD105	RD750	Pks15/1 Δ7bp	RD711	RD702
SNP marker	OxyR C37T ⁹³	Rv3815c G81A ⁵⁸	RpoB T2646G ⁹³	KatG T1388G ⁴ RpoB C3243T ⁹³	Not known	Not known
Geographical association	East Africa, southeast Asia, south India	East Asia, Russia, South Africa	East Africa, north India, Pakistan	Americas, Europe, north Africa, middle east	Ghana, Benin, Nigeria, Cameroon	Senegal, Guinea-Bissau, The Gambia
Comments	Similar to ancestor based on presence of Tbd1 ^{86,92}	Traditionally known as <i>M africanum</i> subtype 1 (Clade 1)	Traditionally known as <i>M africanum</i> subtype 1 (Clade 2)

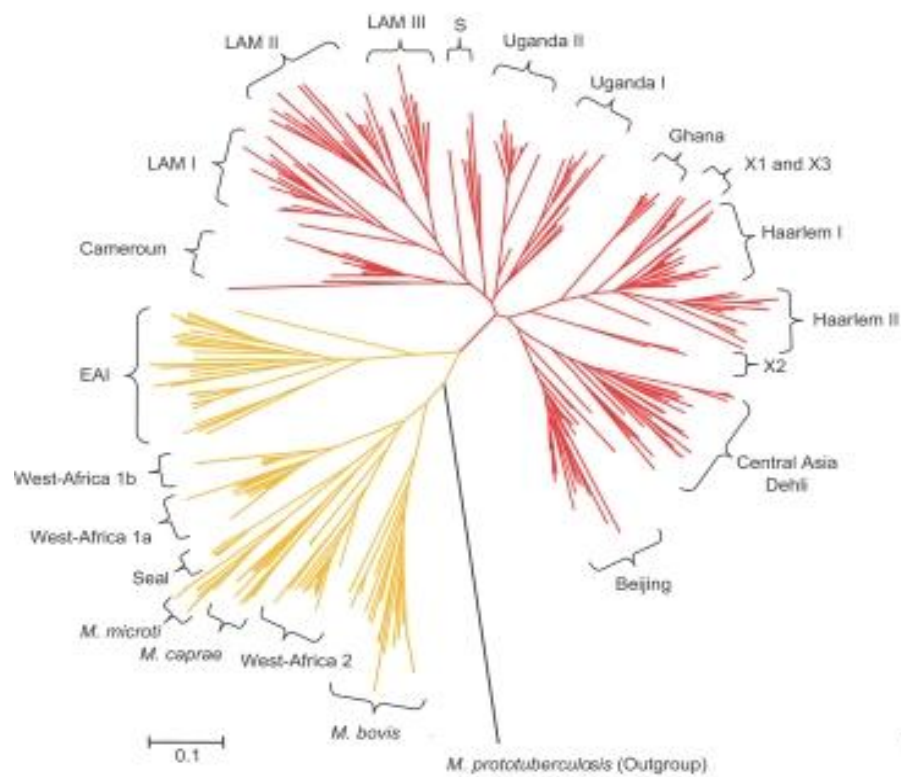


Summary

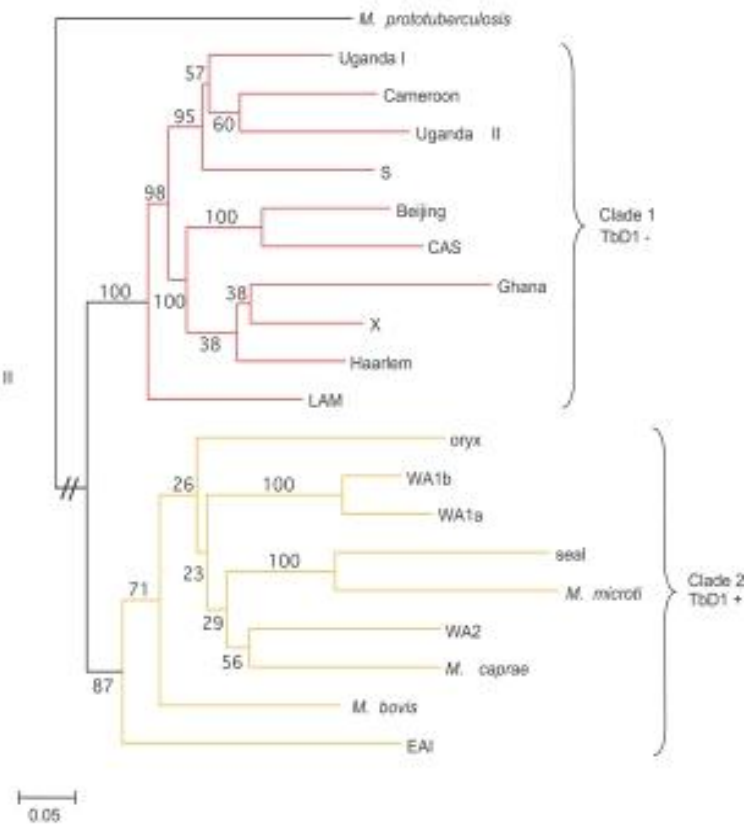
Gagneux et al.

- Complicated classification schemes
- Diagnostic tests, drug and vaccine development affected
 - Phenotypic variability
 - Geographical strain variation

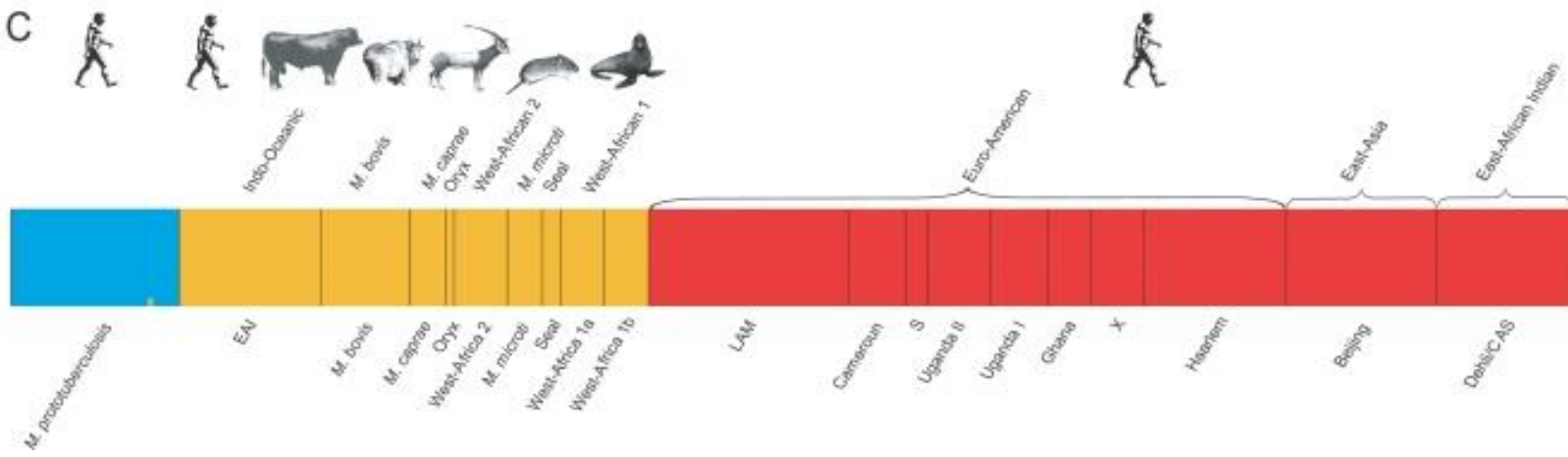
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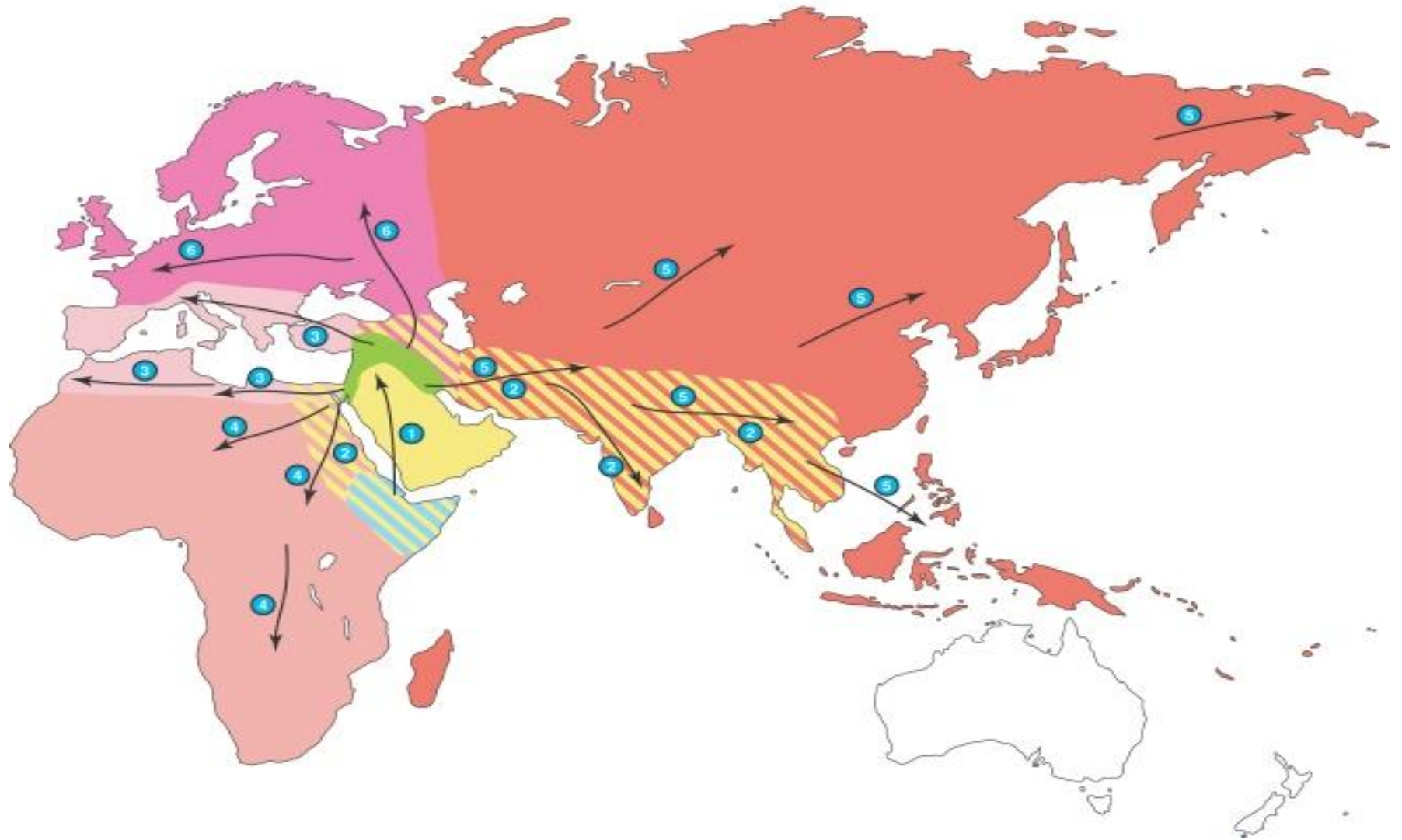
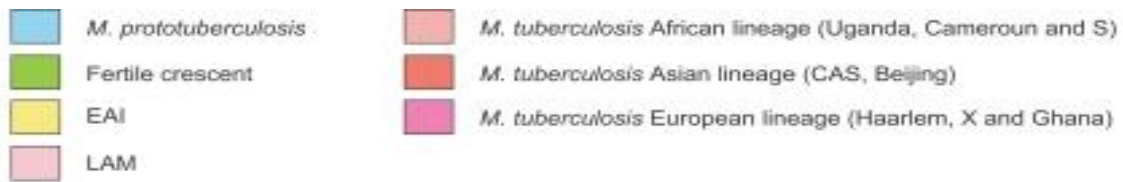


B



C





Summary

Wirth et al.

- MTBC composed of two major lineages
- Emerged ~ 40,000 years ago
- Co-migrated with modern humans out of Africa
- Clade 1 human pathogen
- Clade 2 animal pathogen
- Western urbanization, industrialization, modern intercontinental movements enhanced the expansion of the most successful lineage of tuberculosis, Beijing

References

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