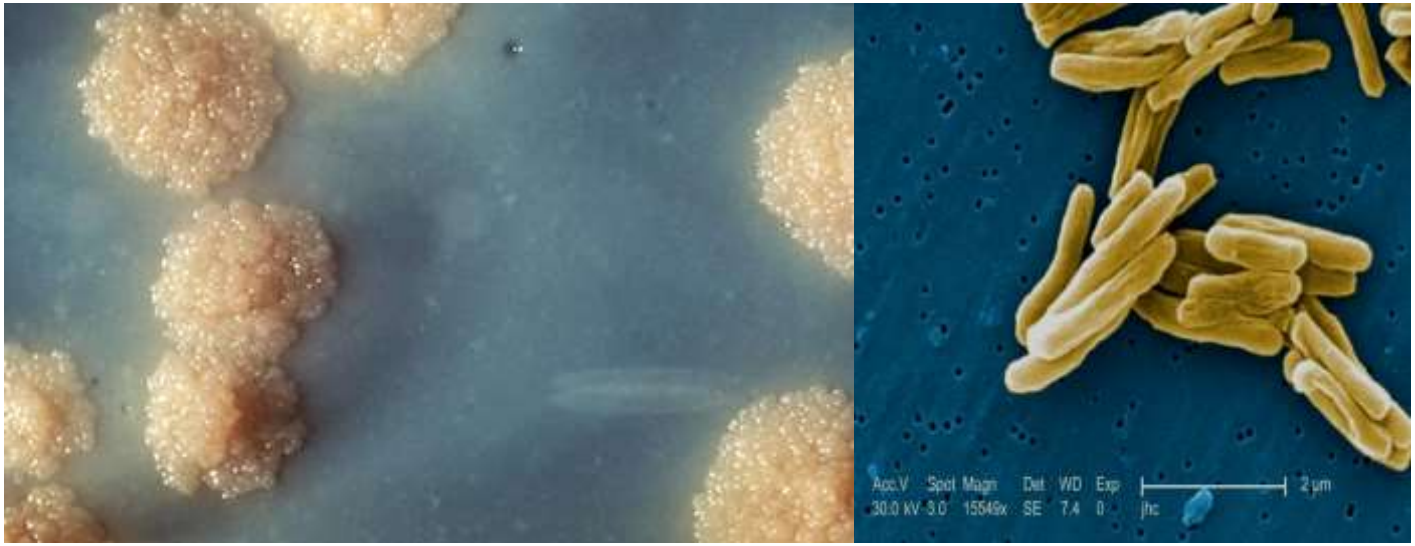

Mycobacterium
tuberculosis

Mycobacterium tuberculosis



Tuberculosis: a Brief history

Tuberculosis is a serious disease and a highly contagious one.
One of the most important and ancient human diseases

- **NEOLITIC**
  - *Mycobacterium Bovis → Mycobacterium Tuberculosis?*
  - First evidences were found in Germany.

- **EGYPT**
  - Mummies dated from 3000 to 2400 b.C
  - First hospital to cure Tuberculosis.
  - Ebers papyrus (1550 b.C) → First description of the disease.

Ebers papyrus

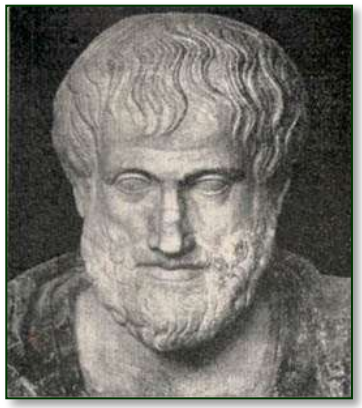
Ajenatón and his wife Nefertiti

Tuberculosis: a Brief history

ANCIENT GREECE

- Hipocrates → *"phthisis"*
- Aristoteles → Transmission of the disease.

Hipocrates



Aristoteles

MIDDLE AGE

- Tuberculosis continued to expand but there was no progress in the understanding of the disease.

XIX CENTURY

- Industrial Revolution → The disease expanded in relation to poverty→ "White Plague"
- From middleage to our days, different scientific advances have been produced.
  - Robert Koch discovered that *Mycobacterium tuberculosis* causes the disease.
  - Different antibiotics and vaccines have been discovered.

TB continues like one of the top three infectious killers worldwide

Epidemiology and global status

- **ACCORDING TO THE WORLD HEALTH ORGANIZATION (2008):**

- TB is a disease associated with the poverty

- Etiologic Agent that produces highest mortality worldwide.

-9 millions of new infections/year

-One third of the world's population is thought to have been infected with *M. tuberculosis, and* 13.7 million chronic active cases.

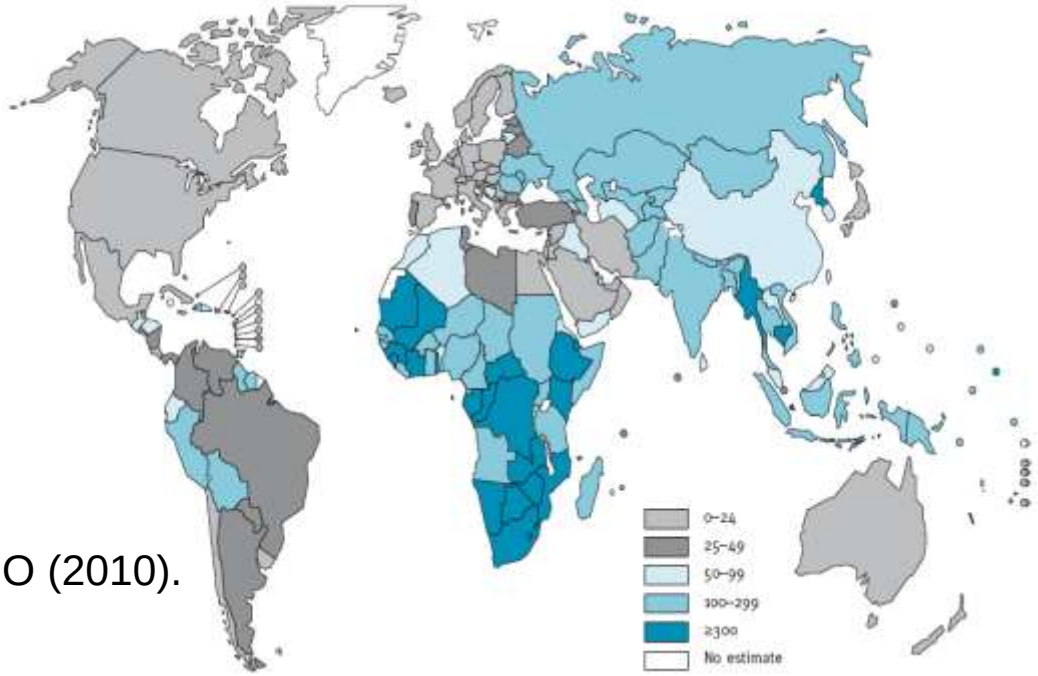
-2 millions deaths/year.

- 1 in every 10 infected people develop TB disease during his lifetime.

Tuberculosis

- *Mycobacterium tuberculosis* complex (*M. tuberculosis, M. bovis, M. microti, M. africanum, M. canetti*)
- 1/3 of the world´s population infected
- 9,3 millions new cases per year
- 1,7 millions of deaths per year
- The most frequent occurrence in developing countries (86 %)
- 900 new cases

  100 deaths per year in CR



Estimated newTB cases
per 100 000 population 2009, WHO (2010).

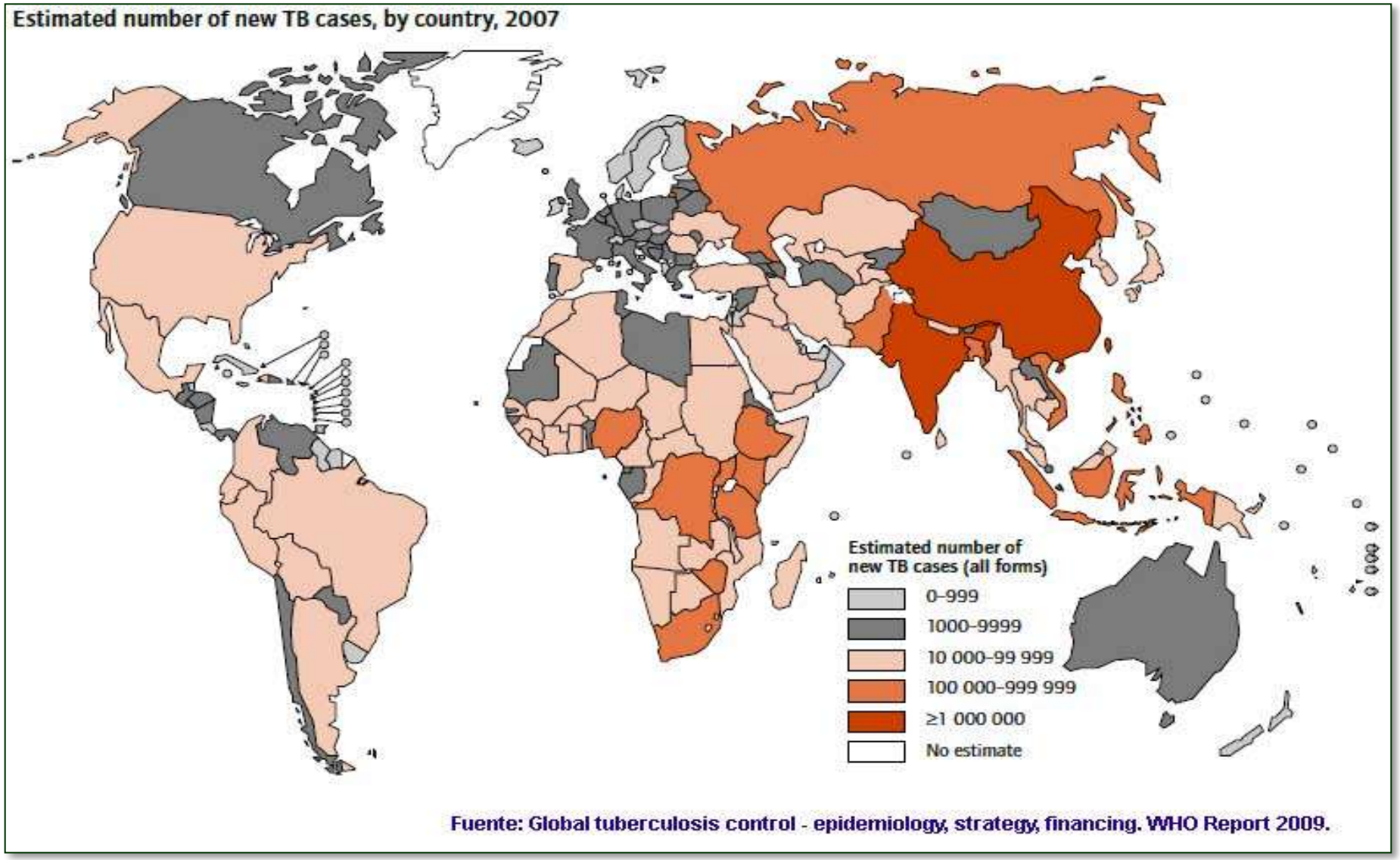
Global status

- Inappropriate control in countries of the third world
- HIV infection
- Inmigration
- Marginalization in developed countries
- Antibiotics-resistant TB

Tuberculosis over the world:

-55% of cases in Asia (India & China → 35% of the total number)
- 30% in Africa
- 7% in Eastern Mediterranean
- 5% in Europe
- 3% in America

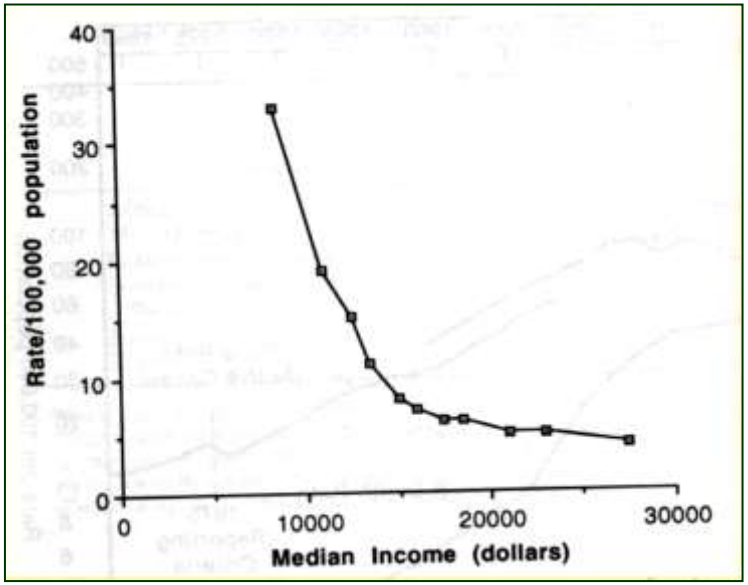
Global status



- **TB still ↑**

- **AFRICA** → adolescents and young adults

- **WESTERN EUROPE AND EEUU**→ Old or immunodeficient people



WE CAN SAY THAT:

1. Tuberculosis kills more people than any other curable disease worldwide.

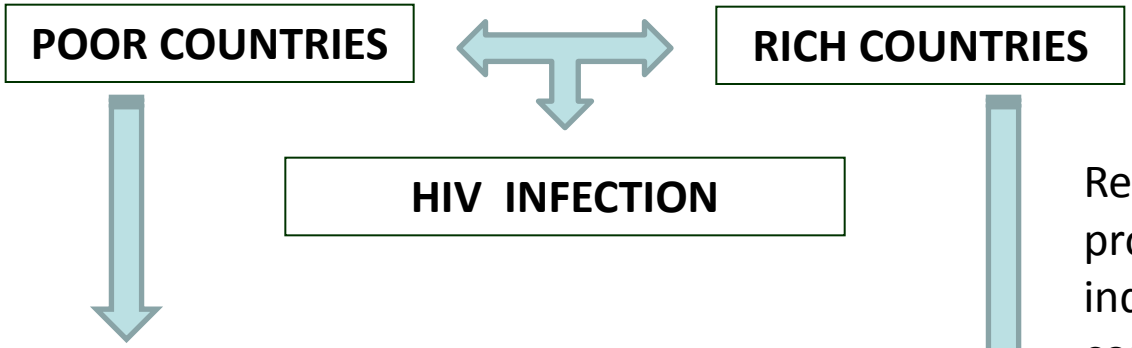
2. A person ill with TB infects approximately 10-15 people per year → every second, a person becomes infected by TB

3. Approximately every minute die 4 people due to tuberculosis.

4. Every day, 25,000 people develop TB and 5,000 die from the disease.

2. Global status

- **CURRENT DATA DONOT CONFIRM A TREND IN THE DECLINE OF TUBERCULOSIS: Tuberculosis is a disease of growing importance especially in conjuction with HIV pandemics – 50% of HIV-positive in SAR have TB…**



- poor nutritional and hygienic conditions
- lack of health resources and their appropriate management

Re-emerging problem in industrialized countries

- Use of immunosupresants
- immigration from countries with high endemic
- increasing resistance of *M. tuberculosis* to drugs

M. tuberculosis H37Rv genom

- Genome 4,4.10^6 bp, 4000 genes

TABLE 1. General classification of *M. tuberculosis* genes

Function	No. of genes	% of total	% of Total coding capacity
Lipid metabolism	225	5.7	9.3
Information pathways	207	5.2	6.1
Cell wall and cell processes	517	13.0	15.5
Stable RNAs	50	1.3	0.2
IS elements and bacteriophages	137	3.4	2.5
PE and PPE proteins	167	4.2	7.1
Intermediary metabolism and respiration	877	22.0	24.6
Regulatory proteins	188	4.7	4.0
Virulence, detoxification and adaptation	91	2.3	2.4
Conserved hypothetical function	911	22.9	18.4
Proteins of unknown function	607	15.3	9.9

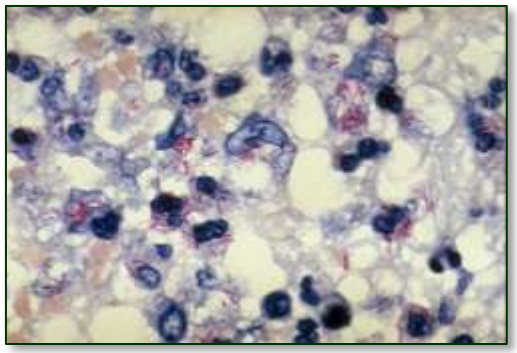
- Over 200 genes related to fatty acid metabolism (*E. coli* 50 genes)
- Unrelated PE (Pro-Glu) and PPE (Pro-Pro-Glu) families of acidic, glycine - rich proteins, may be involved in antigenic variation of *M. tuberculosis* during infection

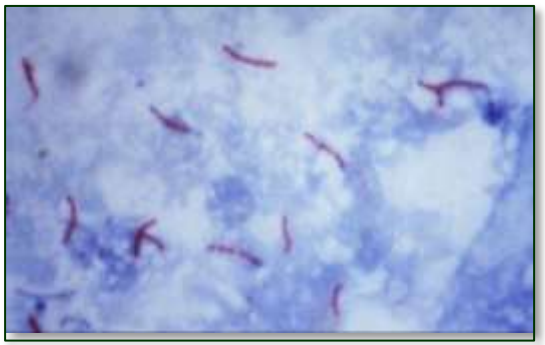
9. Diagnosis

- **Different ways to make the diagnosis of M. Tuberculosis**.

- A complete diagnosis must include several ways of diagnosis, like a medical history, a physical examination, a microbiological examination (of sputum or some other appropriate sample). It may also include a tuberculosis skin-test, other scans and X-rays, surgical biopsy, or other methods (PCR, autofluorescence…).

- **BACILLOSCOPY**
  - Advantages
  - Disadvantages





- **CULTURE (microbiological examination)**

MANTOUX TEST

POSITIVE

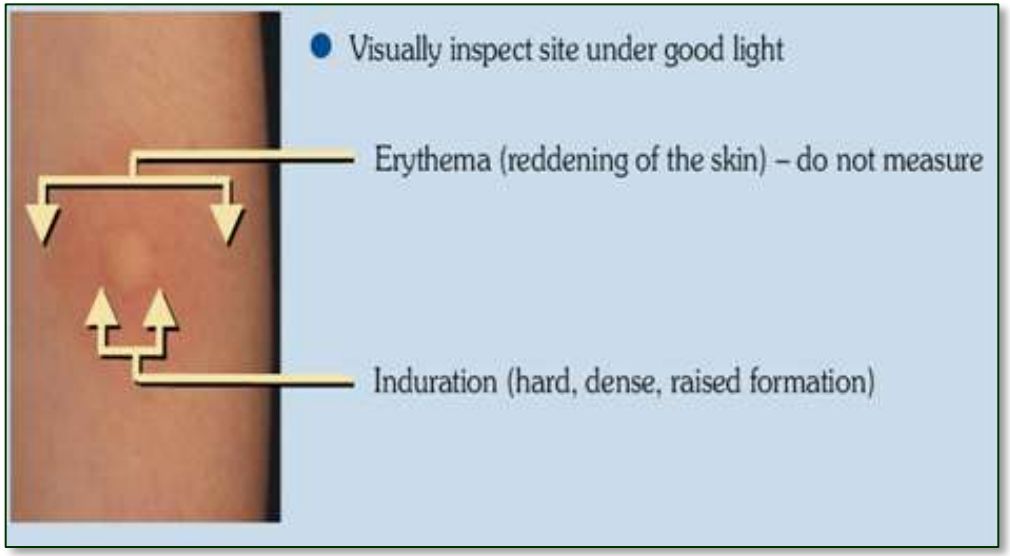
POSITIVE

NEGATIVE

NEGATIVE

MANTOUX REACTION

IF POSITIVE → There has been contact with the bacteria at some point of life



Diagnosis

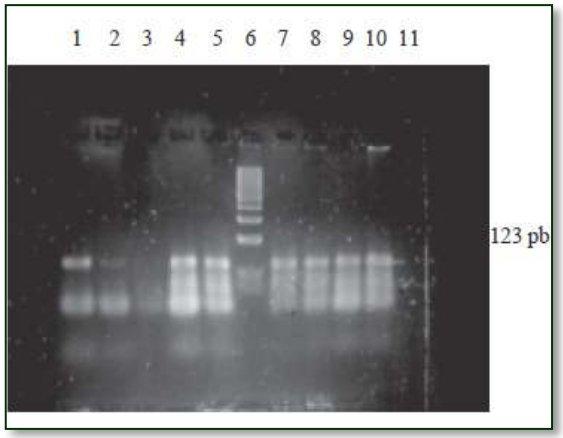
- **PCR** — Direct and fast detection method. 123 Pb IS6110 is a frequently used repetitive sequence.

ADVANTAGES

- Total sensitivy –> 55% - 90%
- Close to 99% specificity.

DISADVANTAGES

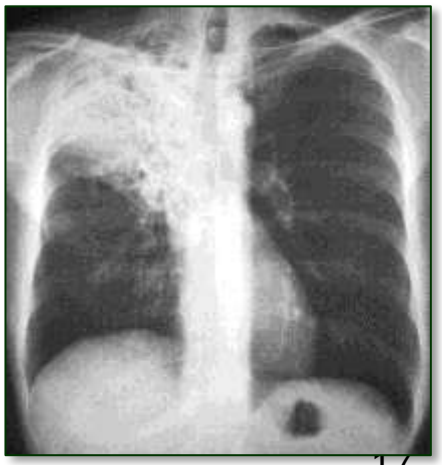
- High cost compared to bacilloscopy and culture.



Detection of MT by PCR

- **X-RAYS** — Very sensitive technique for the diagnosis of pulmonary TB, but completely non-specific.

Radiological injuries highly suggestive of TB



Chest x-ray showing an alveolar infiltration on right upper lobe

Diagnostic of tuberculosis

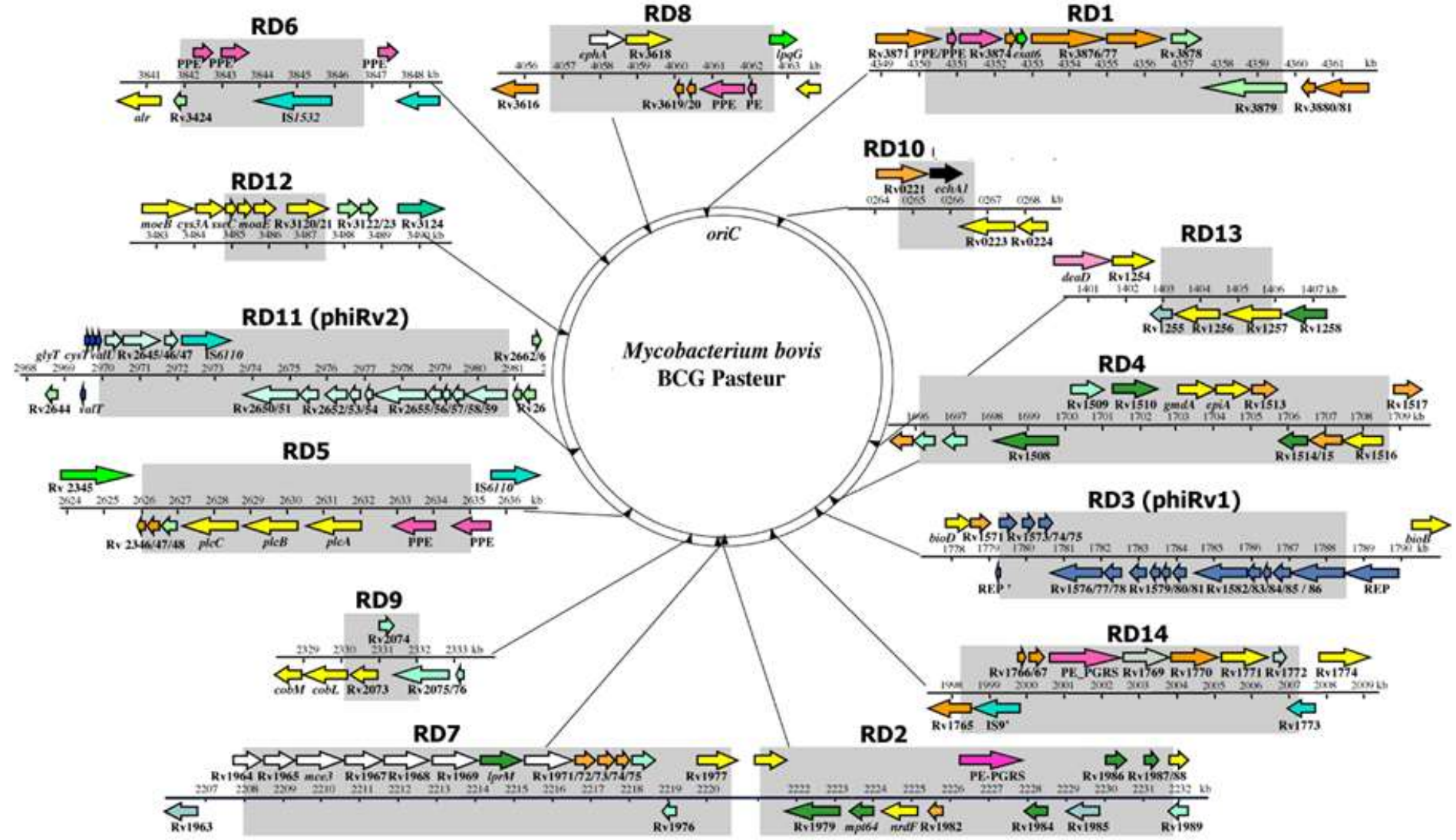
Latent tuberculosis (2 billion people…)

- Tuberculin skin test
  - PPD is intradermally injected, delayed-type of hypersensitivity
  - Crossreactivity with vaccination strain and enviromental mycobacteria
- IGRAs
  - specific T-cell response with IFN-γ release
  - QuantiFERON-TB Gold In Tube (Cellestis)
  - T-SPOT.*TB* (Oxford Immunotec)
  - Fails in immunocompromised individuals, deases influencing immune system, during immunosupresive treatment etc.
  - **Low sensitivity and specificity**

Active tuberculosis

- Sputum smears and cultures – Ziehl-Neelsen staining
- Chest X-ray

Whole genome comparisons of *M. tuberculosis* H37Rv and *M. bovis* BCG

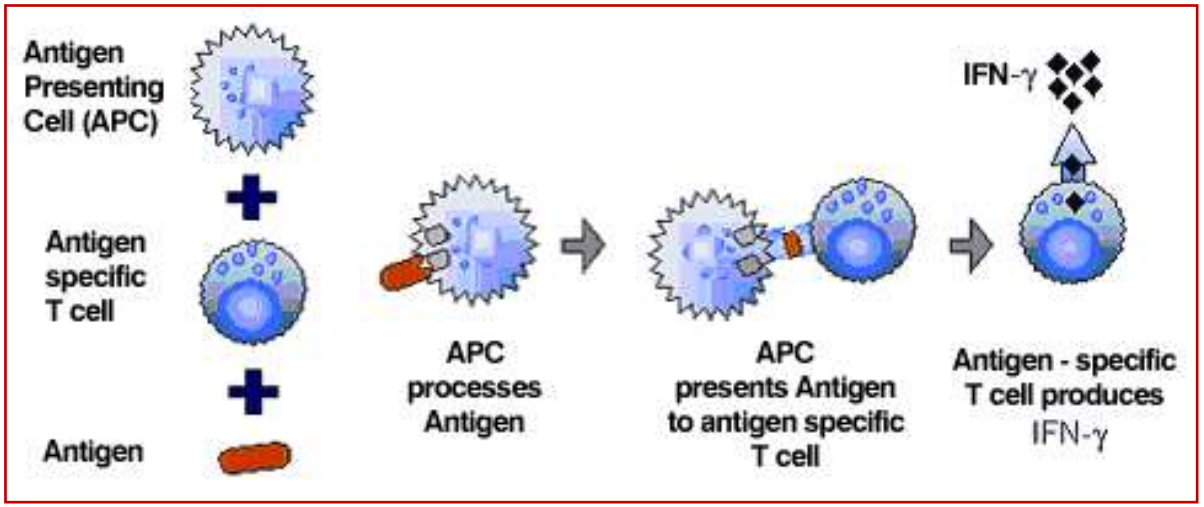


Institut Pasteur *(2000-2005)*

Mycobacterium bovis BCG

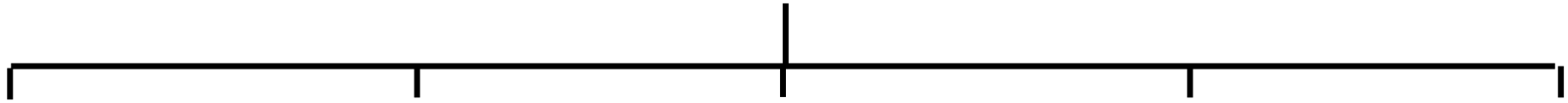
- Live attenuated strain *M. bovis* so-called Bacillus Calmette-Guérin (BCG), vaccine applied to newborns
- Loci RD11, RD1 (*tb7.7, esat-6/cfp-10*) and several others deleted in *M. bovis* BCG and the most of nontuberculosis mycobacteria

Esat-6, CFP-10, Tb7.7 used in tuberculosis diagnostics tests (IGRAs)



Host range of tubercle bacilli

M. tuberculosis complex



M. canettii	*M. tuberculosis*	*M. africanum*	*M. microti*	*M. bovis*
Humans (rare)	Humans (everywhere)	Humans (rare)	Voles Shrews Field mice	Cattle Deer Goats Humans Lions Llamas Seals

99.9 % sequence similarity

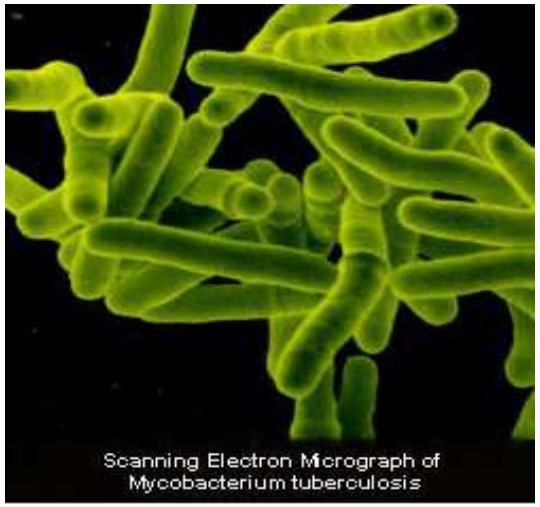
identical 16S rRNA gene sequence

***M. bovis* BCG**

vaccine

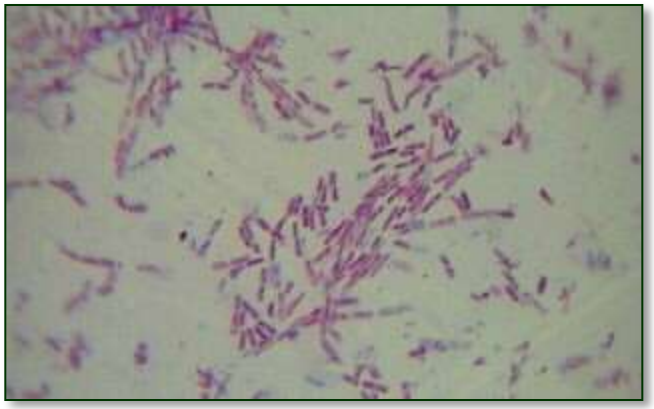
Mycobacterium tuberculosis

- *Actinomycetales, Mycobacteriaceae, Mycobacterium*
- Gram positive – acid fast…
- Obligate aerobe
- Thin rod shape bacteria
- Non-motile
- Non-sporulating? *vs* σ^F
- Generation time 20 – 24 hours
- Acid fastness (after staining resistance to decolorization with acidified alcohol)
- Characteristic - presence of mycolic acid in cell wall

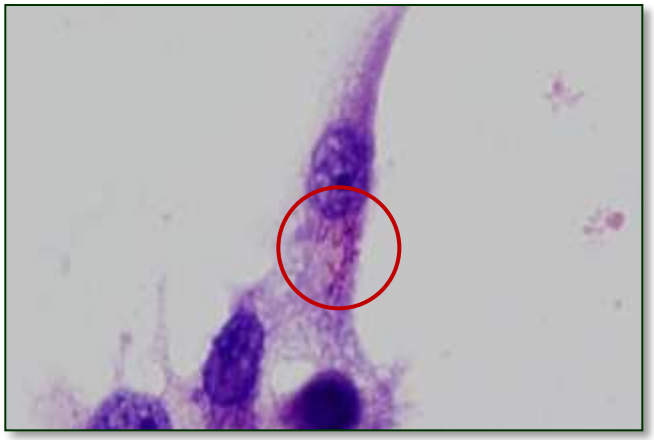


GENERAL CHARACTERISTICS OF *M.Tuberculosis*

- Gram positive or better acidoresistant.
- Non-motil bacteria, no sporulated.
- Cell wall with extremely high lipid content .
- Alcohol resistent acid→ Ziehl Neelsen dye.
- Strictly Aerobic →Uper lventilated lobes of lungs.
- Falcultative, intracelular pathogen (macrofages)
- Latency
- NATURAL RESRVOIR :Human.



M. tuberculosis Ziehl – Neelsen dye.

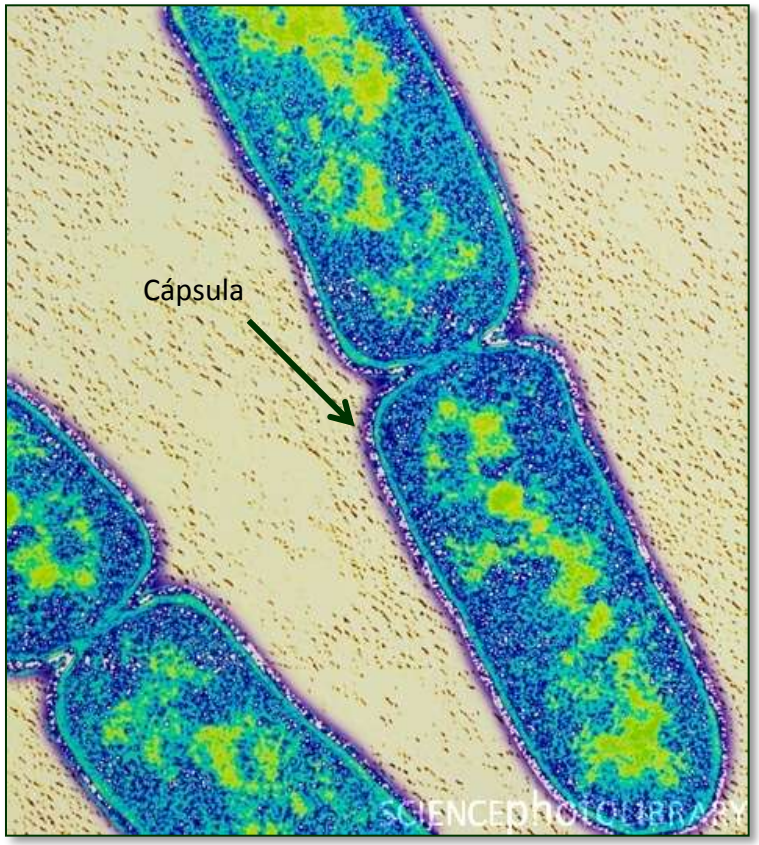


Infected macrofage due to *M. tuberculosis.*

http://inmunidadgabri.blogia.com/2008/021901-mycobacterium-tuberculosis.php
http://www.dicyt.com/viewItem.php?itemId=4811

Bacterial envelope

- **CAPSULE:**
  - External layer of Mycobacteria.
  - Protection against external factors,
  - Principal components:
    - **Mycolic Acid.**
    - **Glycolipids** → responsible of antigenic characteristics and virulences factors.



Mycobacterium tuberculosis

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4. Bacterial envelope

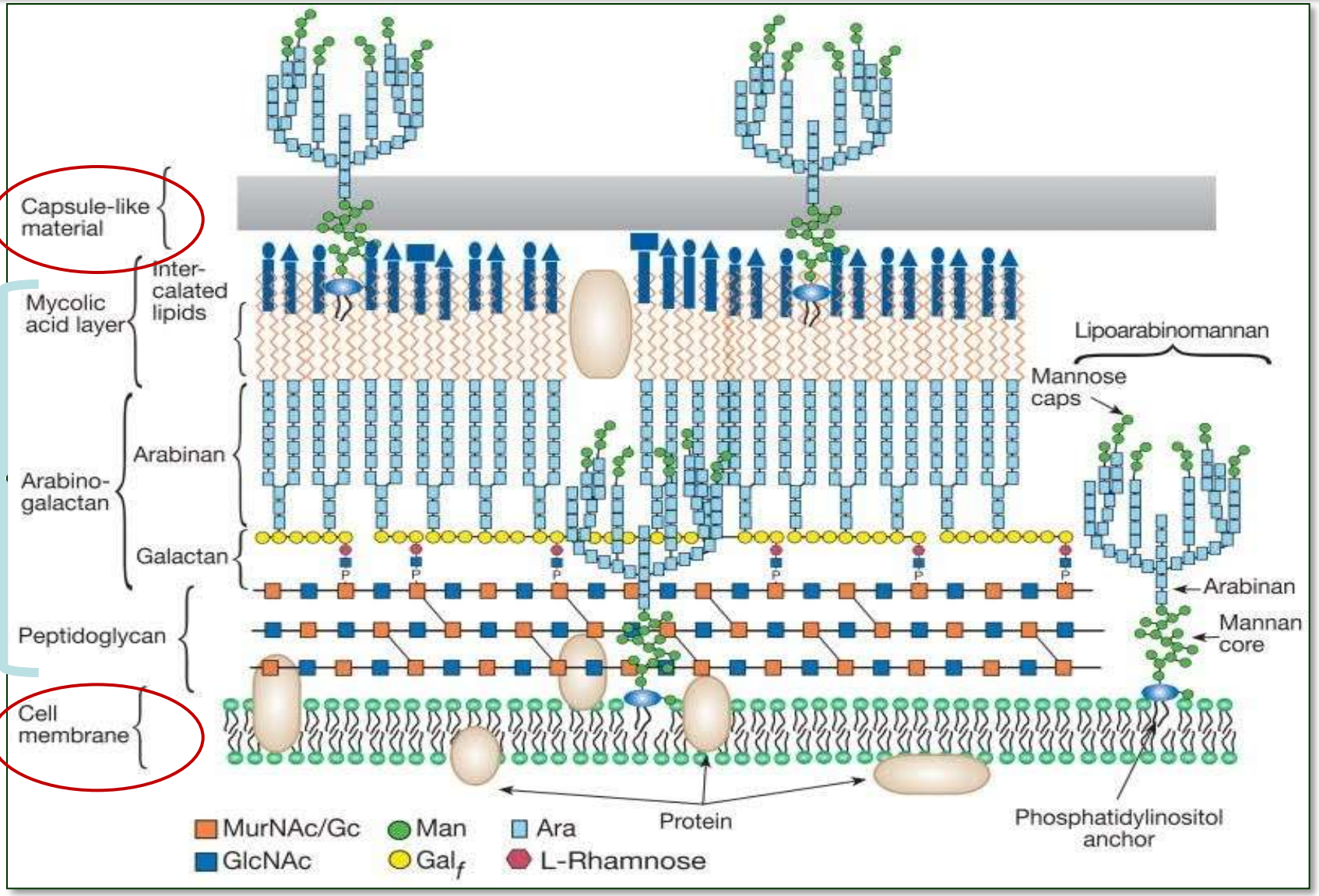
Celular wall

- High lipid content(50-60%)→Hydrofobic and Enzimes lysis resistant.
- Formed by :
  - **Micólic Acids** → TRETAHELOSA BINDED →ANTIGEN (DIM, EVASINS…)
  - **Arabinogalactane**: Complement protection.
  - **Peptidoglycan**: Protect Mycobacterium against osmotic lysis.

CELULAR MEMBRANE:

- **Fosfolípids** highly glicosilates:
  - **Lipoarabinomanane** (LAM): Tuberculosis pathogenesis.
  - **Fosfatidilinositolmanósids** (PIM).

4. Bacterial envelope.



http://3.bp.blogspot.com/_PDPKMAzai6k/TCQVicTrXTI/AAAAAAAAABs/GP6nDjpY0lM/s1600/ch20f8.jpg

VIRULENCE FACTORS OF *M.Tuberculosis.*

- What is a Virulence factor:

A strategic factor contributing to pathogenity in the infected host.

MTB virulence factor classes:

1. MTB is a champion of immune modulation
2. Survival in phagocytes.
3. Avoidance of activated macrophages response.
4. Stimulation of destructive inflamatory response.
5. Factors affecting host susceptibility.

ESX-1 Secretion System

The ESX-1 Secretion System

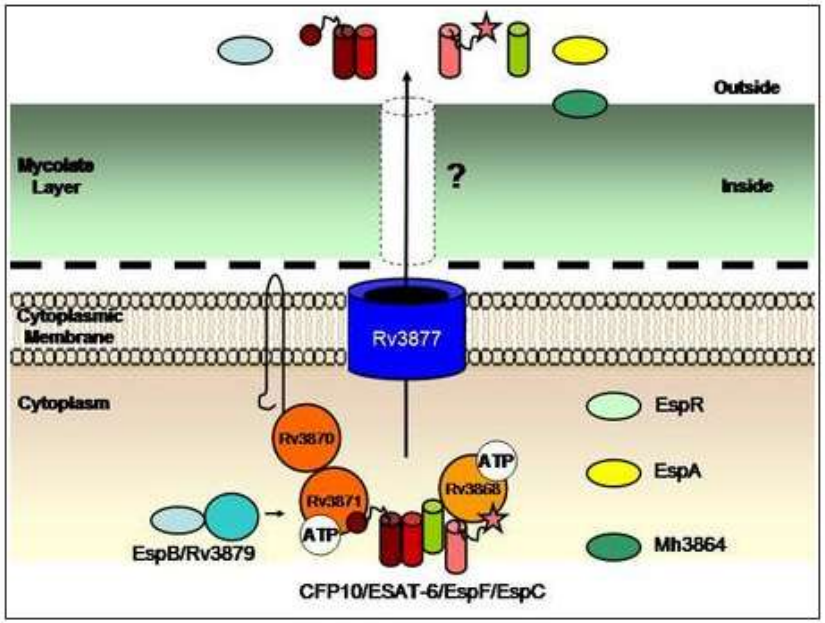


Figure 1: The ESX-1 secretion machine translocates virulence factors across the mycobacterial cytoplasmic membrane. Rv3877 is a multi-transmembrane protein that likely contributes to the formation of a trans-membrane pore. There are three AAA ATPases associated with ESX-1, including Rv3870, Rv3871 and Rv3868, which likely provide energy for secretion. Substrates include the CFP-10/ESAT-6 pair, EspC, EspF, EspA, EspB, EspR and Mh3864. Our work supports a model in which C-terminal regions of ESX -1substrates function to target them to cognate ATPases, either directly or through protein interaction with other substrates. The CFP-10 signal sequence targets substrates to Rv3871, while the C-terminal amino acids of EspC targets substrates to Rv3868. One possibility is that prior to or after the formation of a multi-substrate complex (likely including CFP-10, ESAT-6, EspF and EspC), engagement of the C-termini by the ESX-1-associated ATPases activates the machine for secretion. EspB likely is indirectly recognized through Rv3879c by Rv3871 (McLaughlin et al., 2007), while EspA (Fortune et. al. 2005) is likely targeted through Rv3868, although the mechanism by which this occurs is unknown thus far. Mh3864 (Carlsson et al., 2009) and EspR (Raghavan et. al.,2008) are also secreted by ESX-1, but the way that these substrates are targeted remains unknown.

Mycobacterium tuberculosis
Important T cell antigens – toxins?

- Culture filtrate proteins (serum)

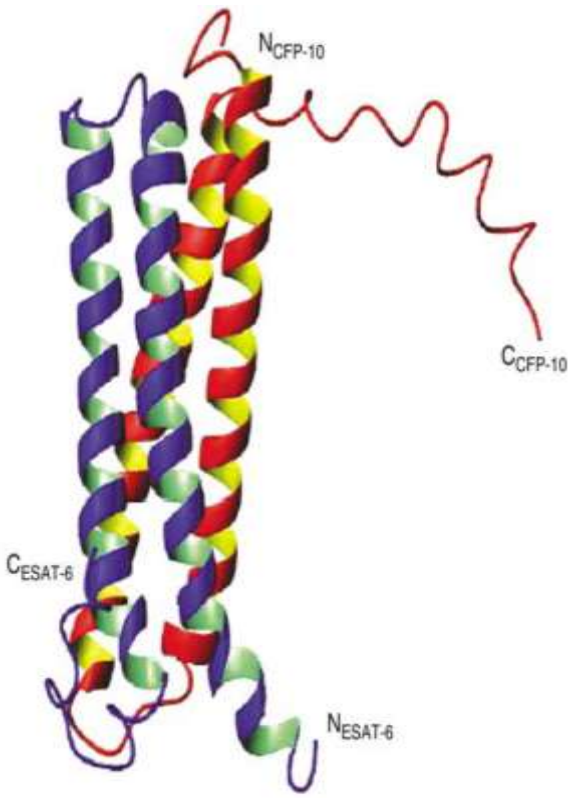
Esat-6/CFP-10

  - *esat-6/cfp-10* (Rv3875, Rv3874) in locus RD1
  - tight **1:1 complex**
  - **potent inducers** of Th-1 cytokines
  - C-terminus of CFP-10 is essential for **binding** to the surface of cells
  - C-terminal 6 AA of ESAT-6 can **bind to macrophage surface** Toll-like receptor 2
  - CFP-10/ESAT-6 are secreted by **type VII** (ESX-1) secretion system which is essential in TB pathogenesis

Acr1

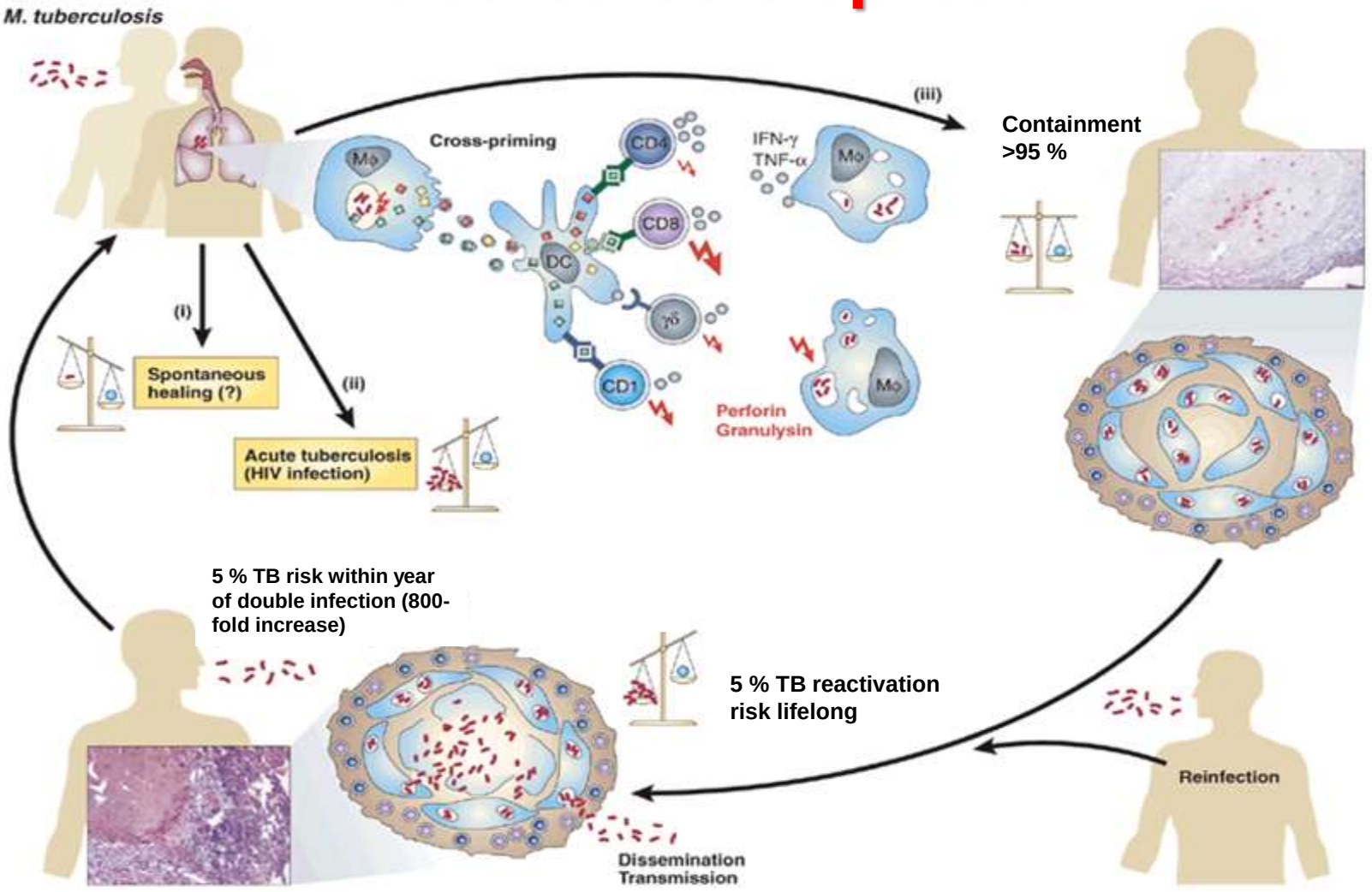
  - *acr1* Rv2031c , α-crystalline
  - Serum of TB patients, induced under anoxic condition

- Tb7.7
  - Rv2654 locus RD11, C-terminus induces strong T cell immune response in TB patients



Renshaw P. S. *et al*.: *EMBO J* 24 (2005)

Tuberculosis spread



Adapted Kaufmann S.H.E. *et al*.: *Nature Medicine* 11 (2005).

Different outcomes of *M. tuberculosis* infection and underlying immune mechanisms. *M. tuberculosis* enters the host within inhaled droplets. Three outcomes are possible. (**i**) Immediate eradication of *M. tuberculosis* by the pulmonary immune system. This alternative is rare to absent. (**ii**) Infection transforms into tuberculosis. This frequently occurs in immunodeficient individuals, with the notable example of HIV infection increasing the risk of developing tuberculosis 800-fold. (**iii**) Infection does not transform into disease because *M. tuberculosis* is contained inside granulomas.

M. tuberculosis vs host interaction

Initial infection

- Respiratory tract - bronchial epithelium produces antimicrobial peptides with a wide spectrum of activity
- Replication and dissemination of the pathogen are restricted by mononuclear phagocytes
- T cells are recruited to the site of primary infection containing the bacilli

Mycobacterial dormant state and persistance

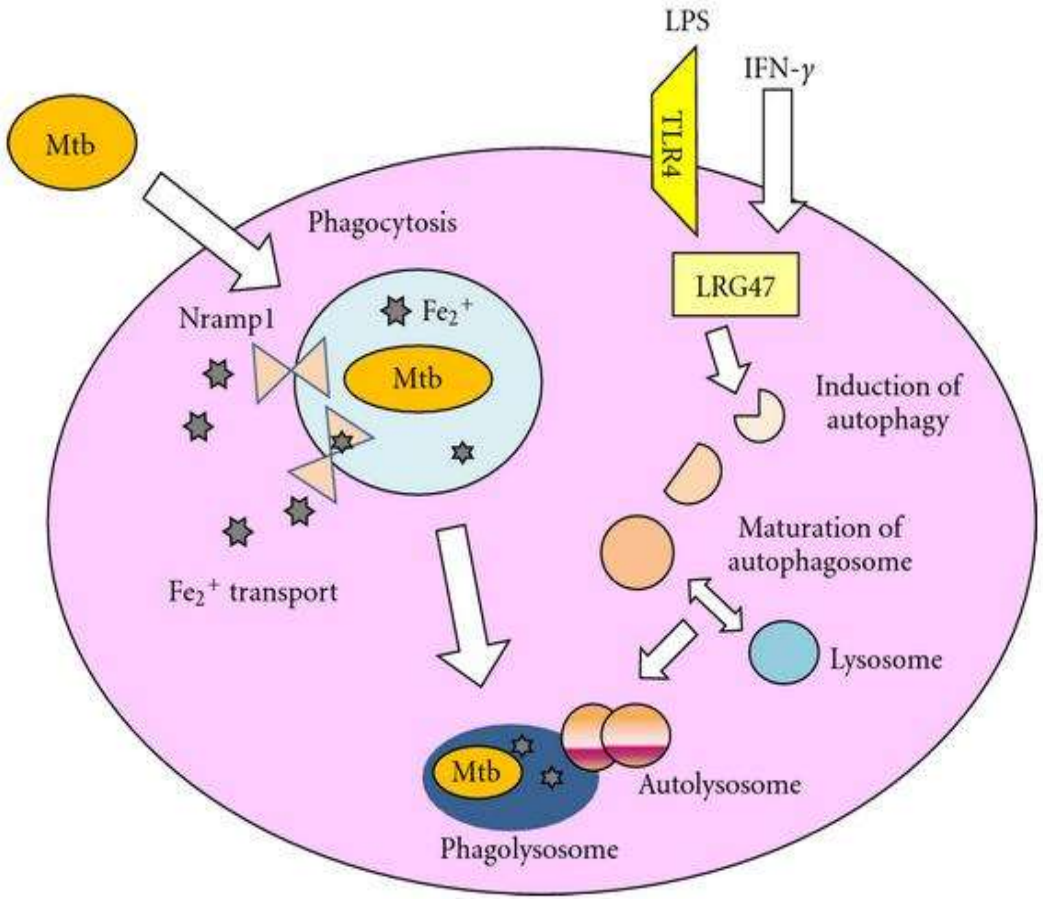
- Avoid direct confrontation with the host immune defense
- *M. tuberculosis* further retards its replication rate
- Granuloma formation
- Spores formation???

Reactivation of the bacilli

- Aging, malnutrition, steroids or HIV, drugs…

4. Factors affecting host susceptibility

- **Nramp1**: Natural resistance associated macrophage protein



Nramp1 is expressed in the phagosomal membrane and mediates mycobacterial killing by sequestering iron uptake.

LRG47stimulates autophagy in macrophages, responsible for mycobacterial killing by promoting fusion of mycobacterial phagosomes to lysosomes.

Progression of the disease

- M.Tuberculosis infects alveolar macrophages → It's able to survive and multiply in the inactivated ones.

CD4+ T cells ⇒ Macrophages activation

- Infected inactivated macrophage

CD8+ T cells — Kill infected macrophages that can not destroy the bacteria

- **The progression of the disease depends on the ability of a person to mount a rapid and effective activated macrophages response.**

Spread and progression of Tuberculosis

- **Transmission:** The disease is spread by aerosols, and is a highly contagius one.

- A person is infected if is skin-test positive → 1% of this people develop the active disease (sometimes high)

- **Symtops:**
  - **Lung TB:** Fever, coughing , loss of energy and weight, progressive and irreversible lung destruction.

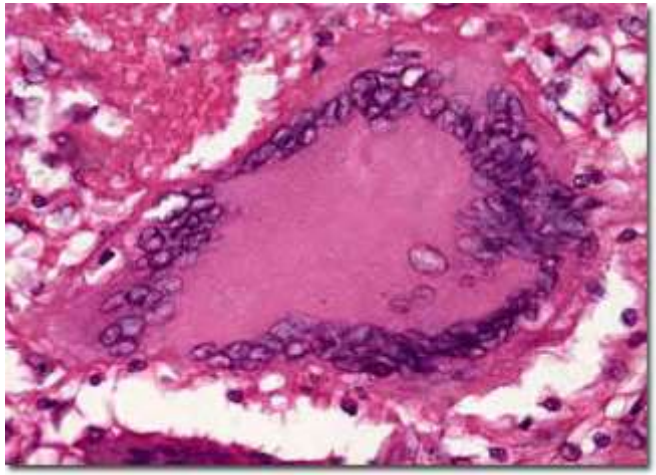
  - **Systematic TB:** It's almost always fatal.

Progression of the disease

- Since the phagocytic cells are not clearing the infection, new Tcells and macrophages continue to be attracted and accumulate around the sites where bacteria are growing, trying uncessfully to kill the bacteria.

GRANULOMATOUS RESPONSE
(Tcell mediated)

GRANULOMA FORMATION

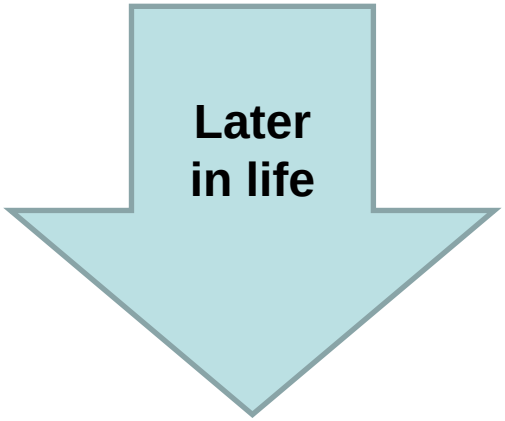


- Caseus necrosis
- Damage to the lung

-Bacteria cease to grow → Lesion calcifies
- Bacteria continue growing → Lesion liquefies

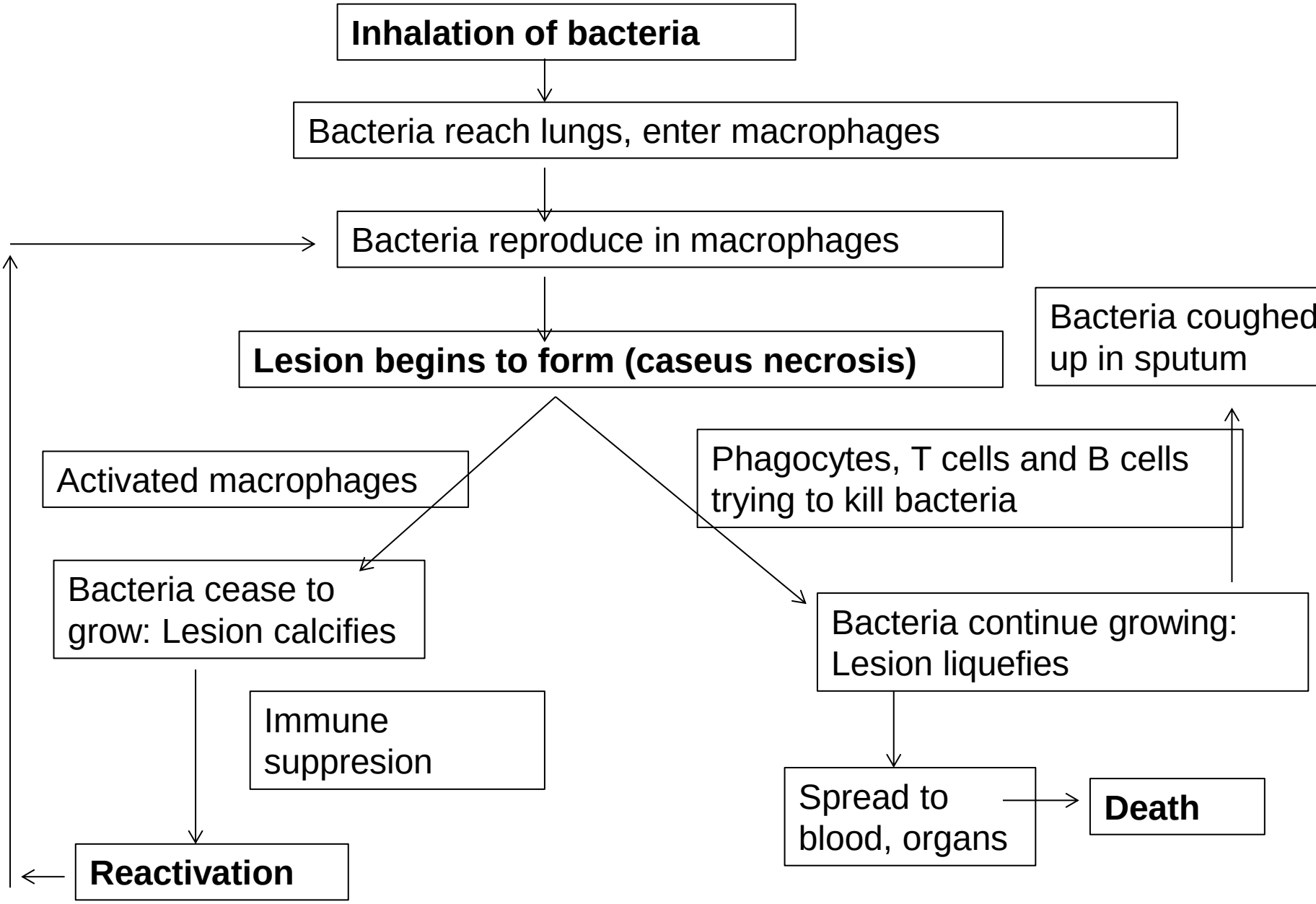
Progression of the disease

- **If the patient can not kill the MTB, the bacterium has the ability to survive for decades in such lesions.**



Supression of the immune system

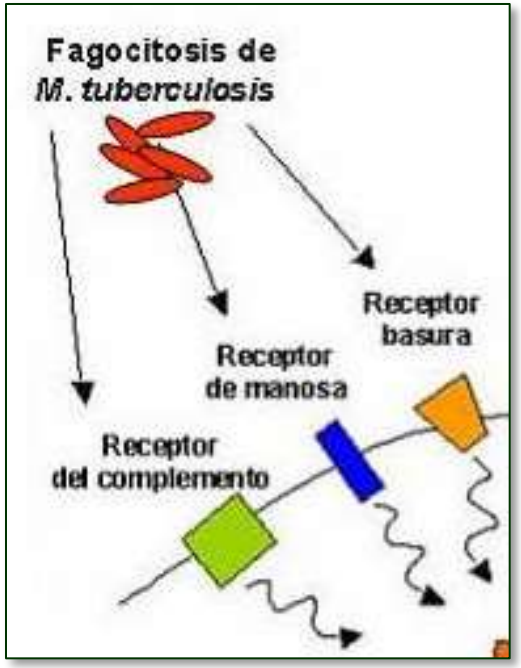
Reactivation TB
(bacteria break out the lesion and begin to multiply again)


Inhalation of bacteria
Bacteria reach lungs, enter macrophages
Bacteria reproduce in macrophages
Lesion begins to form (caseus necrosis)
Bacteria coughed up in sputum
Activated macrophages
Phagocytes, T cells and B cells trying to kill bacteria
Bacteria cease to grow: Lesion calcifies
Bacteria continue growing: Lesion liquefies
Immune suppresion
Spread to blood, organs
Death
Reactivation

Entry into phagocytes

- A key virulence property of M.Tuberculosis is its ability to survive and multiply inside monocytes and macrophages.
- **To entry into them is necesary to:**

1. Binds { CR3 / CR4 } → Bacteria internalized into macrophages by a vesicle.



RECEPTORS:

- **Manosa receptors.:**
- **Receptors for the Fc** (Ig): opsonization.
- **Complement receptors** CR1 y CR3/CR4
- ***Scavenger*-A receptors :**

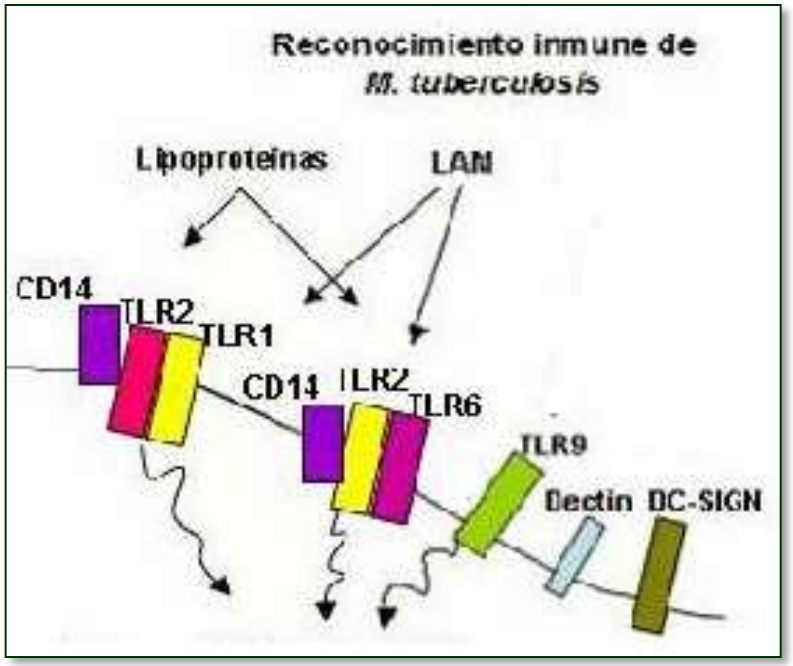
Immune response

- **INNATE IMMUNE RESPONSE**
  - **Alveolar macrophages (AM) → First line of defense**
  - **MTB → RECOGNITION by TLRs:**

LAM + Other molecules → RECEPTOR **TLR-2**

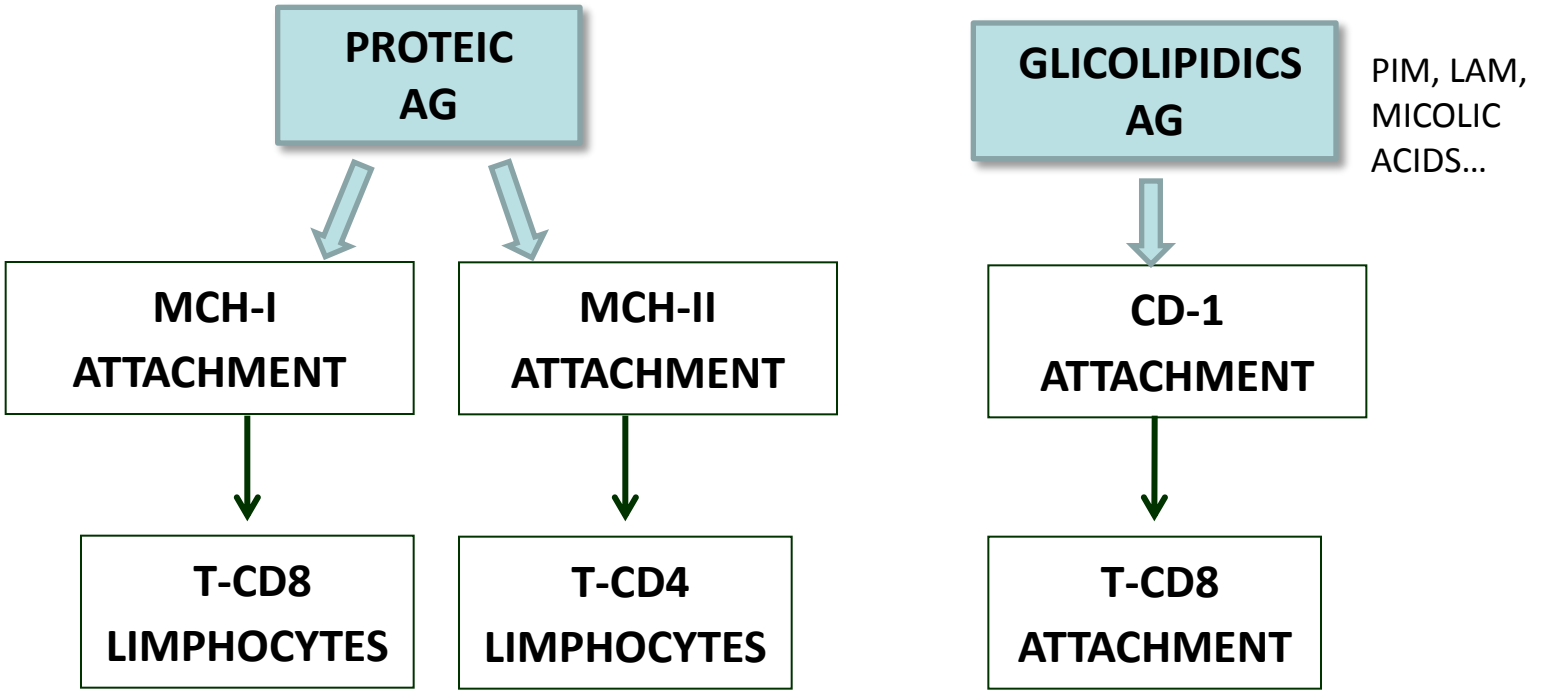
- INTERLEUKIN-12 (**IL-12**)
- TUMOR NECROSIS FACTOR α (**TNF-α**)
- NITRIC OXID (**NO**)

→ **ADAPTIVE IMMUNE RESPONSE**



Immune response:

- **INNATE IMMUNE RESPONSE:** **WITHIN THE PHAGOSOME→ Bacteria still releasing peptides and antigenic molecules**



INNATE IMMUNE RESPONSE → ADAPTATIVE IMMUNE RESPONSE

Ag Recognition, celular types activation + **citokines and chemokines**

7. Immune response

ADAPTATIVE IMMUNE RESPONSE

- **Citokine production → Tcells and NK cells are recruited.**
- **Complex signal network**

IL-12 → **T-LYNPHOCYTES + NK CELLS** → **IFN-γ:**

FUNCTIONS:

1) Induction of IFN-γ production
2) Proliferation of TCD4+ lymphocytes
3) Increased cytotoxicity CD8+ T cells and NK cells.

IFN-γ → **ACTIVATION OF ALVEOLAR MACROPHAGES**

FUNCTIONS

1) Phagosoma acidification + phagosome-lysosome fusion
2) **Transferin**decreases → Fe limits bacteria growth
3) Increases the expression of MHC-I + MHC-II

7. Immune response

ADAPTATIVE IMMUNE RESPONSE:

- **CYTOKINES PRODUCTION → recruit T and NK cells.**
- **Complex signal network.**

TNF-α → PRODUCED BY AM + Tcells → RECRUITMENT OF INFLAMMATORY CELLS

FUNCTIONS:

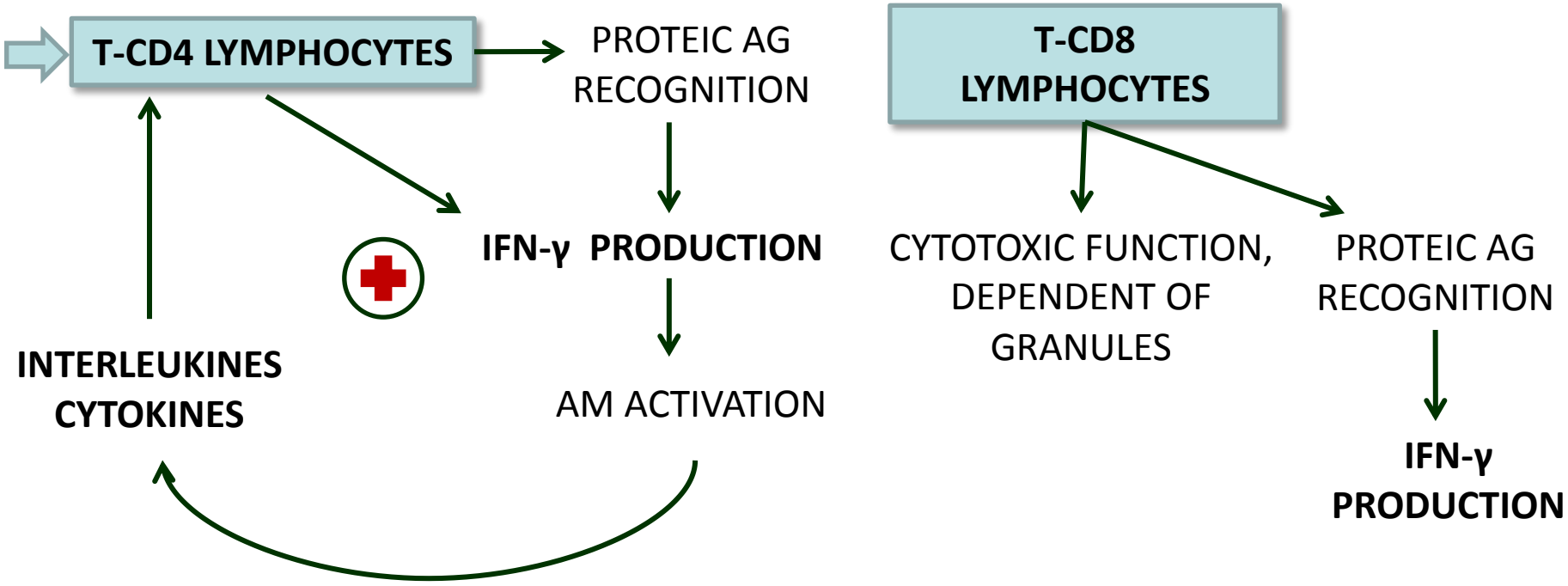
1) Multiple mechanisms of the immune response
2) Stimulation of the acute phase of the local inflammatory reaction and tissue destruction.

CHEMOKINES → ATTRACTION OF IMMUNE SYSTEM CELLS TO THE INFECTION AREA

7. Immune response

ADAPTATIVE IMMUNE RESPONSE

- **CYTOKINES PRODUCTION → recruit T and NK cells.**
- **Complex signal network and cell types network.**



Survival in phagocytes.

- **To survive in macrophage it is necessary to:**

Block vesicle acidification

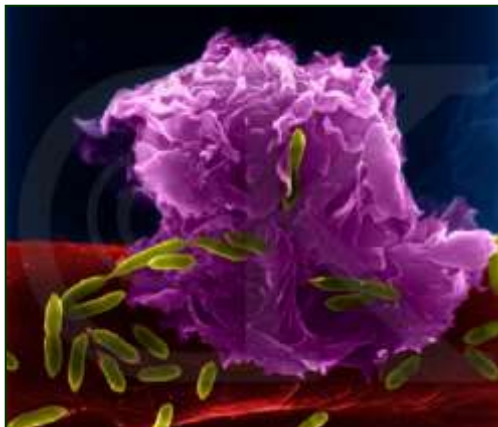
→ Fagolysosome is not formed.

Suppress :

- Oxidative burst.
- IL-12 production → Supression of Th1 response.

4. Phenolic glycolipids from bacteria cell wall :

→ Protect it from ROS



2. Avoidance of the activated macrophages response

LAM (lipoarabinomannan).

- Suppress T cel proliferation.

→ -Reduce IL-2 Production by macrophages.

- Block expresion of MHCII in macrophages.

- Block iINFγ Transcripctional factors.

→ - Thus prevent INFγ from triggering macrophages activation..

Ag 85 change immune system response.

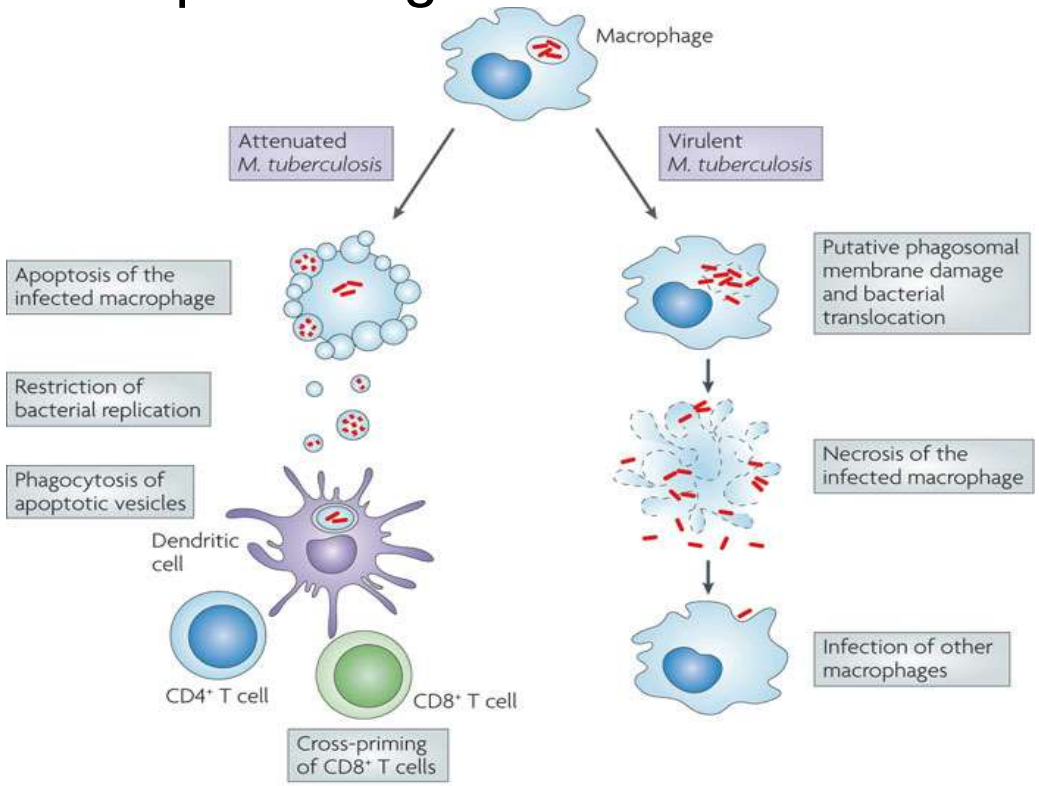
- From protective response to a non productive one.

Stimulation of destructive systemic inflammatory response

- **Antigen 85:** Protein identified that can evoke a skin test response.
- **Mycolic acids** (cell wall component)
- **Muramyl dipeptid** → Stimulate the immune system and trigger proinflammatory cytokine production.
- **TNF-α** → Stimulate local inflammation and tissues destruction (causing an important damage to the lungs).
- Releasing of **toxic lysosomals components** by macrophages trying to inggest and kill the bacteria contribute to lung damage.

M. tuberculosis vs host interaction

Macrophage – mycobacterium interactions in the host response against tuberculosis



Nature Reviews | Microbiology

MACROPHAGE-MYCOBACTERIUM INTERACTIONS IN THE HOST RESPONSE AGAINST TUBERCULOSIS

I. Surface binding of *M. tuberculosis* to the macrophage
   - Complement receptors CR1, CR3, CR4
   - Mannose receptors
   - Surfactant protein receptors
   - CD14
   - Scavenger receptors

II. Phagosome-lysosome fusion

III. Mycobacterial growth inhibition and/or killing
   - Production of reactive nitrogen species
   - Production of reactive oxygen species
   - Apoptosis

IV. Recruitment of accessory immune cells and development of a local inflammatory response
   - Elaboration of cytokines, e.g., TNF-α
   - Elaboration of chemokines, e.g., IL-8
   - Antigen presentation

Schluger N. W.: *Am J Respir Crit Care Med* 157 (1998).

Mycobacterium **tuberculosis infects macrophages** - survives and replicates in the phagosome.

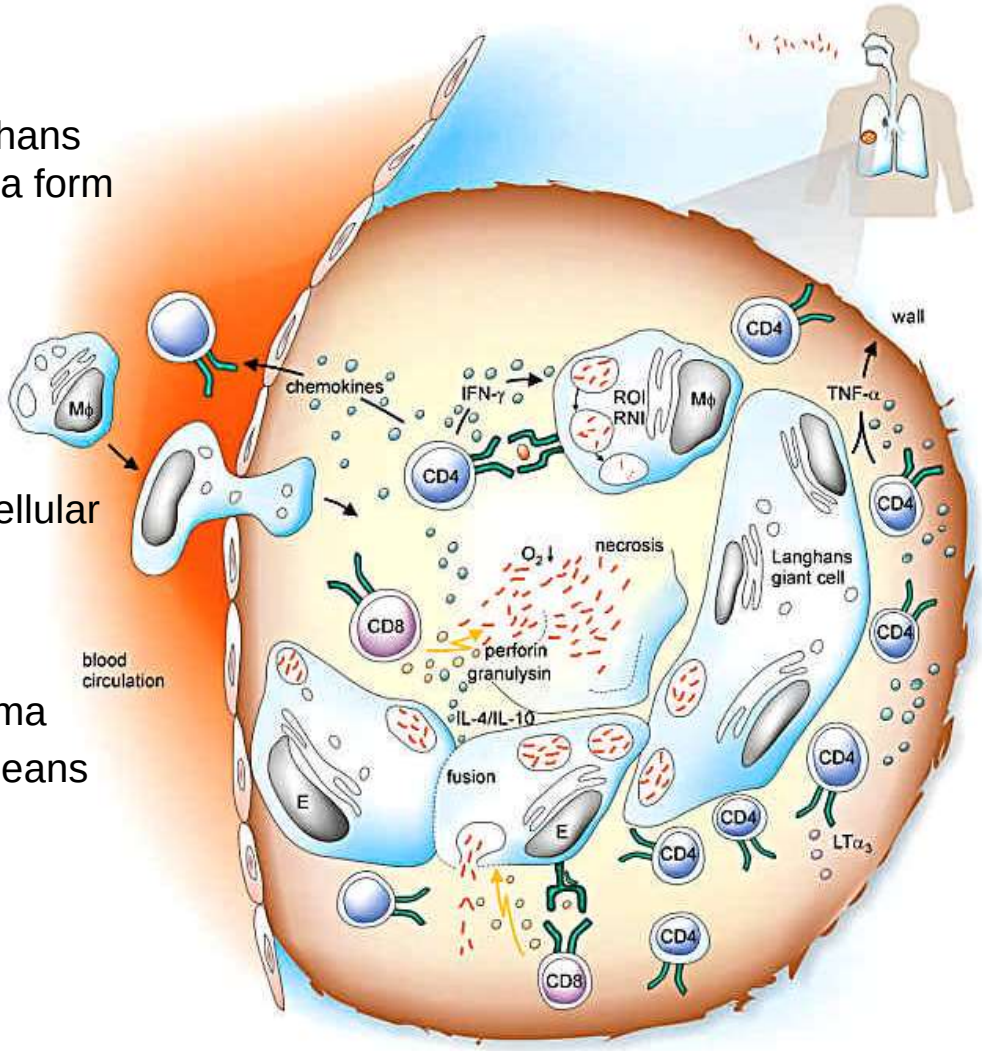
- Macrophages infected with **attenuated strains of *M. tuberculosis* undergo apoptosis**, a death modality that impairs bacterial replication. Apoptotic vesicles containing bacterial antigens are taken up by dendritic cells. The dendritic cells can present these antigens to naive T cells, leading to their activation.
- **Virulent *M. tuberculosis* inhibits apoptosis and induces necrosis**. Damage to the phagosomal membrane facilitates bacterial translocation into the cytosol and is a precursor to the full-scale induction of macrophage necrosis. Necrosis leads to intercellular dissemination of *M. tuberculosis*.

M. tuberculosis vs host interaction

Host factors involved in persistence and latency

Host response and granuloma formation

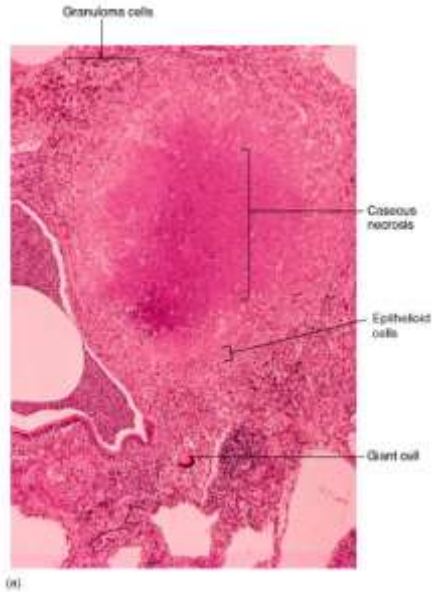
- Alveolar macrophages, epitheloid cells, Langhans giant cells harboring intracellular mycobacteria form the center of the granuloma
- Antigens presenting to T cells
- Chemokines recruit additional cells from blood to the site of primary infection
- $CD4^+$ T cells produce IFN-γ activating macrophages and other APC to kill the intracellular bacteria via reactive oxygen intermediates or reactive nitrogen intermediates
- $CD4^+$ T cells produce TNF-α, lymphotoxin α, formation of the wall surrounding the granuloma
- Activated $CD8^+$ T cells kill mycobacteria by means of granulysin and perforin

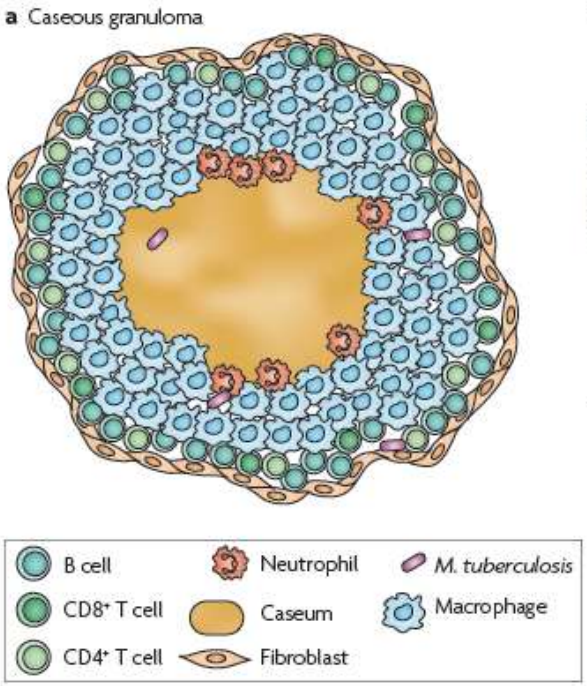


Ulrichs T. *et a*l.: *Frontiers in Bioscience* 7 (2002).

M. tuberculosis vs host interaction

Tuberculous granulomas





Barry C. E. *et al.*:Nature Reviews/Microbiology 7 (2009).

a The classic tuberculous granuloma in active disease, or LTBI. Epithelial macrophages, neutrophils, and lymphocytes (CD4+ and CD8+ T cells, B cells). Mycobacteria is in macrophages in the **necrotic hypoxic centre**.

b The non-necrotizing granuloma, active disease, macrophages, lymphocytes. *M. tuberculosis* is within macrophages in this lesion.

c Fibrotic lesions LTBI, active disease, composed almost completely of fibroblasts, minimal of macrophages.

Granuloma formation host protection (restrict mycobacterial growth)
vs mycobacteria induces granuloma formation

M. tuberculosis vs host interaction

Tuberculous granuloma induction via interaction of a bacterial sectered protein with host epithelium

Volkman H. E. *et al*.: *Science* 22 (2010).

- *Mycobacterium marinum* in zebrafish induces granulomas
- Protein Esat-6 induces metalloproteinase-9 (MMP9)
- MMP9 enhances recruitment of macrofages contributing to granuloma maturation

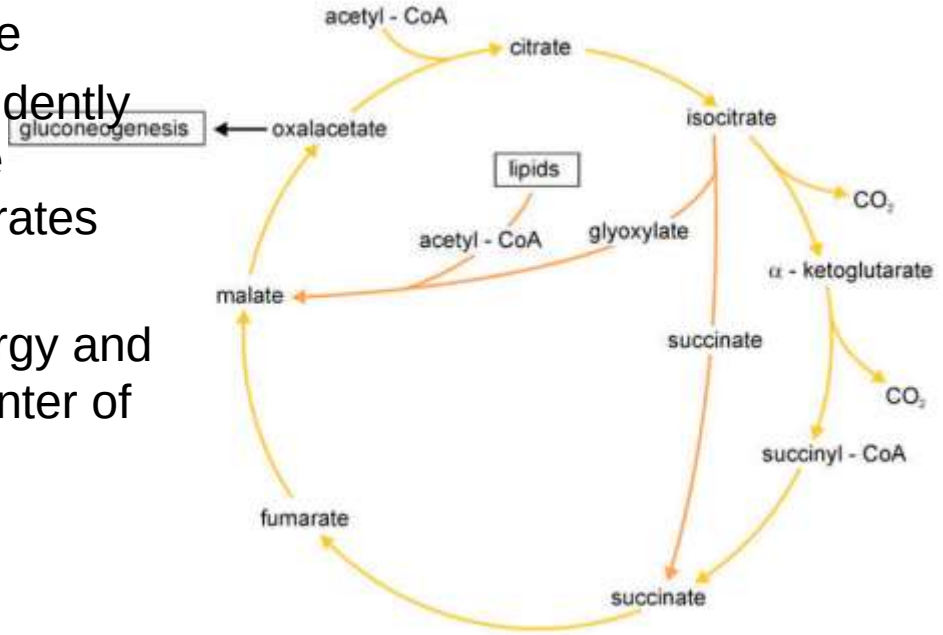
early granuloma facilitates mycobacterial growth

M. tuberculosis vs host interaction

Mycobacterial enzymes involved in persistence

Low oxygen content adaptation within granuloma

- **Upregulation of glyoxylate shunt enzymes**
  - Isocitrate lyase and malate synthase
  - Allows to generate glucose independently from oxygenconsuming steps of the conventional synthesis of carbohydrates (citrate cycle)
  - Advantage – usage of lipids as energy and metabolic source in the caseous center of granulomas



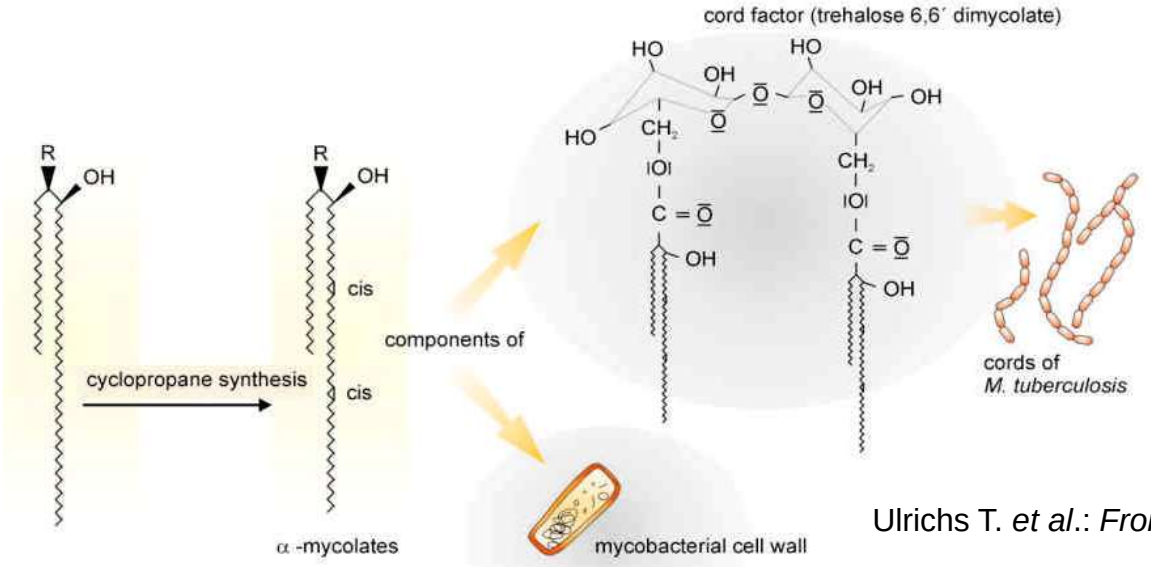
Ulrichs T. *et a*l.: *Frontiers in Bioscience* 7 (2002).

M. tuberculosis vs host interaction

Mycobacterial enzymes involved in persistence

Low oxygen content adaptation within granuloma

- Nitrate reductase
  - Nitrate as electron acceptor instead of oxygen respiration
- Cyclopropane synthase
  - Modifying mycolic acids by cyclopropanating the proximal end
  - Defect in mycolic acid synthesis alters the outer surface, affecting membrane fluidity, permeability and antigenicity



Cord-factor, a virulence factor which allows mycobacteria to form long cords

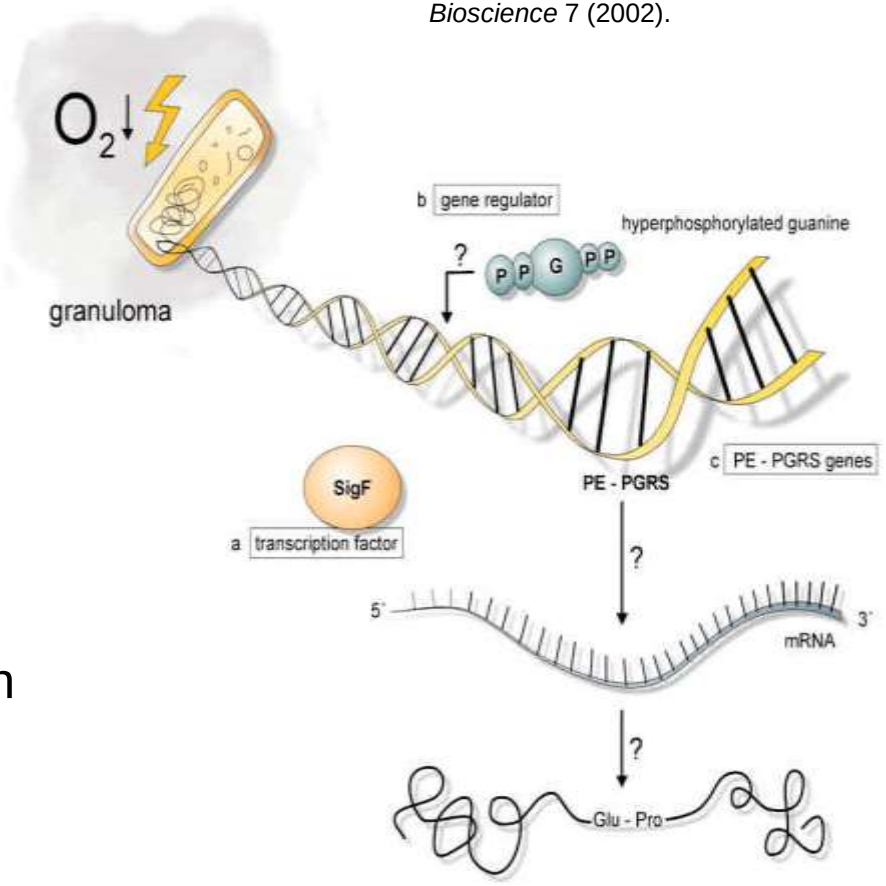
Ulrichs T. *et al*.: *Frontiers in Bioscience* 7 (2002).

M. tuberculosis vs host interaction

Mycobacterial genes involved in persistence

- Factor σ^F
  - Related to sigma factor in *Streptomyces coelicolor* and *Bacillus subtilis*
  - Direct the transcription machinery to distinct genes required for bacterial survival under altered conditions
- Hyperphosphorylated guanine
  - Serves as gene regulator under starvation conditions
- PE-PGRS genes
  - The repeat PE-PGRS is shared by approximately 60 genes of *M. tuberculosis* which encode glycine-rich proteins with a characteristic Glu-Pro-containing motif

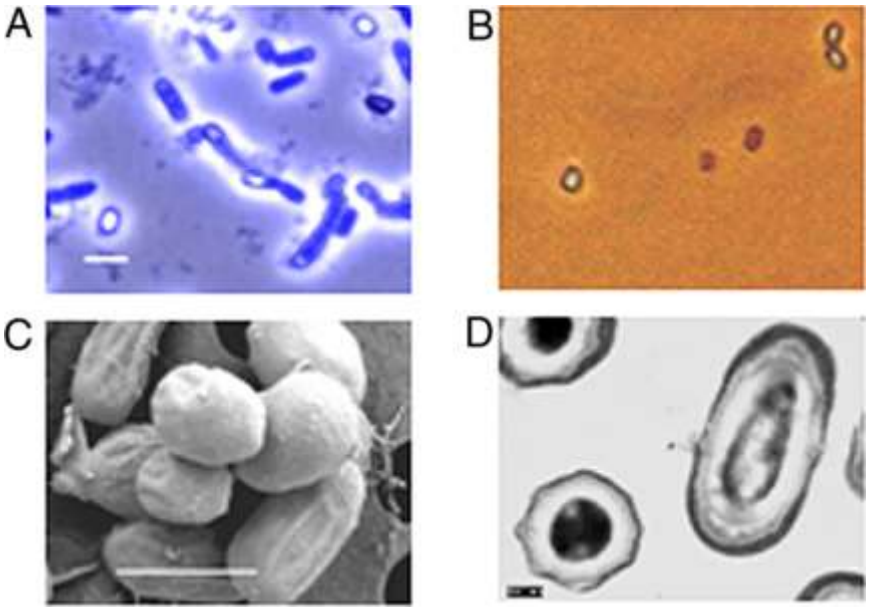
Ulrichs T. *et al*.: *Frontiers in Bioscience* 7 (2002).



M. tuberculosis vs host interaction

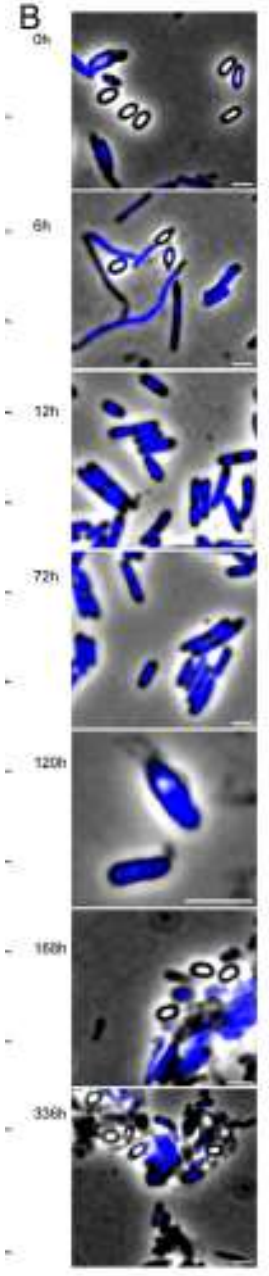
Sporulation of mycobacteria?

- New data indicate that also old *Mycobacterium bovis* BCG cultures form spores?
- Sporulation as a lifestyle adapted by mycobacteria under stress



M. bovis BCG
6-month-old culture

Ghosh J. *et al*: *PNAS* 106 (2009).

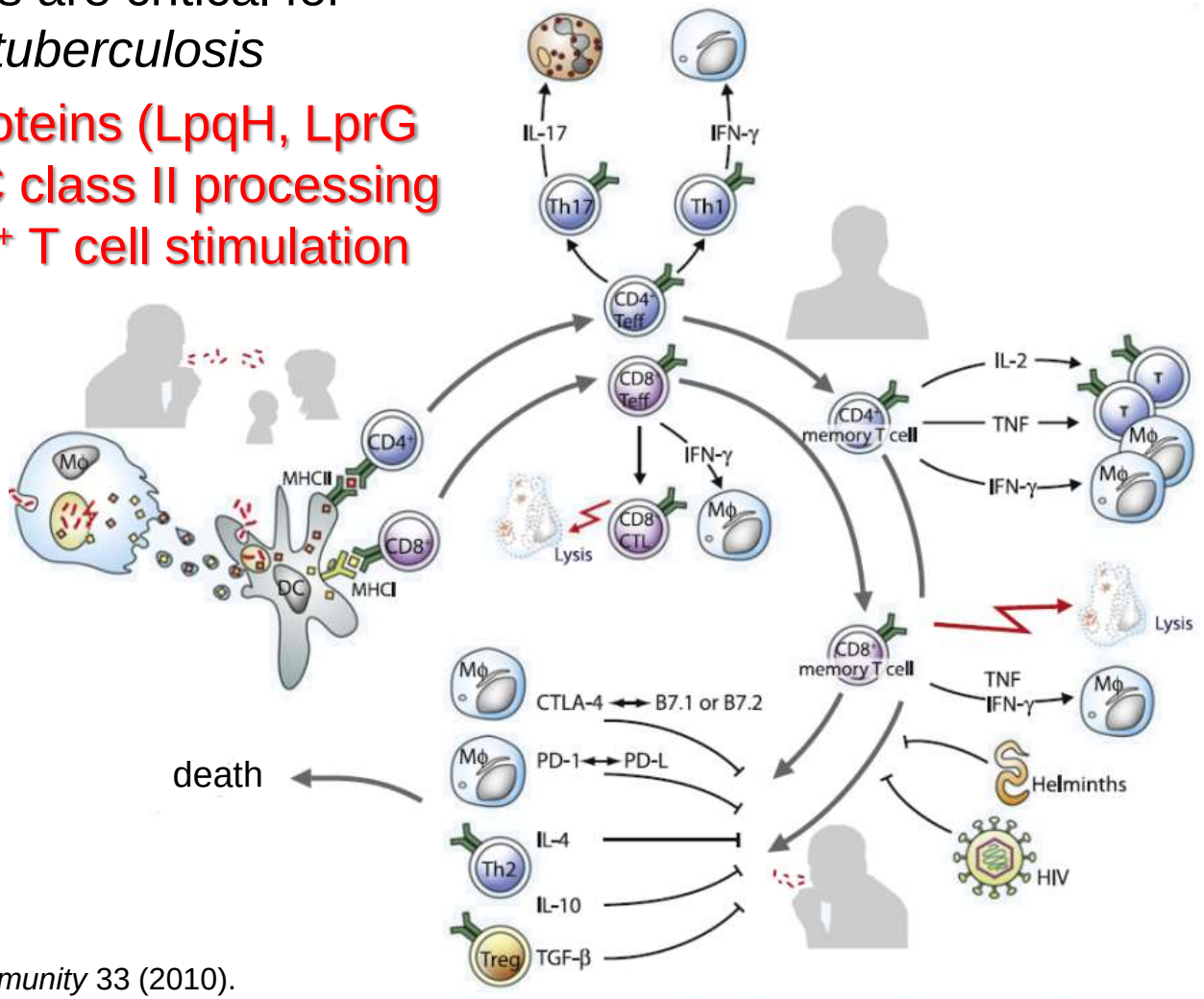


Mycobacterium marinum spores

M. tuberculosis vs host interaction

The Pathologic and Cellular Basis of the Host Response in TB

- $CD4^+$ and $CD8^+$ T cells are critical for protection against *M. tuberculosis*
- *M. tuberculosis* lipoproteins (LpqH, LprG and LprA) inhibit MHC class II processing and thus impairs $CD4^+$ T cell stimulation



Kaufmann S.H.E.: *Immunity* 33 (2010).

M. tuberculosis vs host interaction

Cytokines IFN-γ and TNF-α

- $CD4^+$ T cells - the main production of cytokines in tuberculosis
  - Dramatic increase in susceptibility to tuberculosis of patients infected with HIV (loss of $CD4^+$ T cells)
  - IFN-γ is critical in the control of *M. tuberculosis*, mice deficient in IFN-γ highly susceptible to mycobacteria
- TNF-α has important role in granuloma formation and prevents endogenous reactivation by modulating cytokine levels and limiting histopathology
  - Mice deficient in TNF-α exhibited poorly formed granulomas with areas of extensive necrosis
  - **Patients treated by anti-TNF-α drugs are at risk of tuberculosis reactivation**

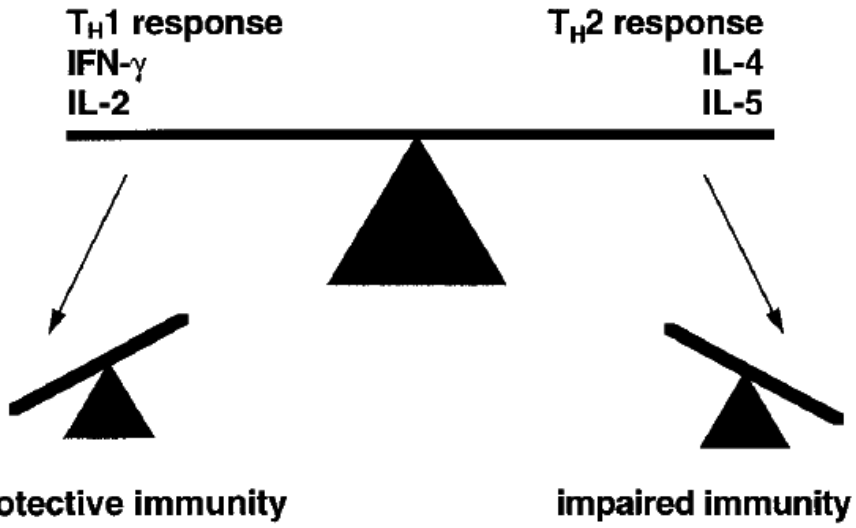
Collins H. L. *et al*.: *Immunology* 103 (2001).

M. tuberculosis vs host interaction

affecting the Immune system balance

- $CD4^+$ Th1 lymphocytes secrete IFN-γ, capable of activating other inflammatory and phagocytic cells
- $CD4^+$ Th2 phenotype secrete interleukin-4 and interleukin-5, cytokines that are involved in recruitment of eosinphils and production of IgE
- Th1 reactions typically characterize protective immunity
- Th2 reactions often represent impaired immunity

Pro-inflammatory response



Anti-inflammatory response

Schluger N.W. *et al*.: *Am J Respir Crit Care Med* 157 (1998).

Treatment

- **TREATMENT:**
  - It is based on the existence of ≠ bacilars populations in the tuberculous focus.

ASPECTS OF TREATMENT

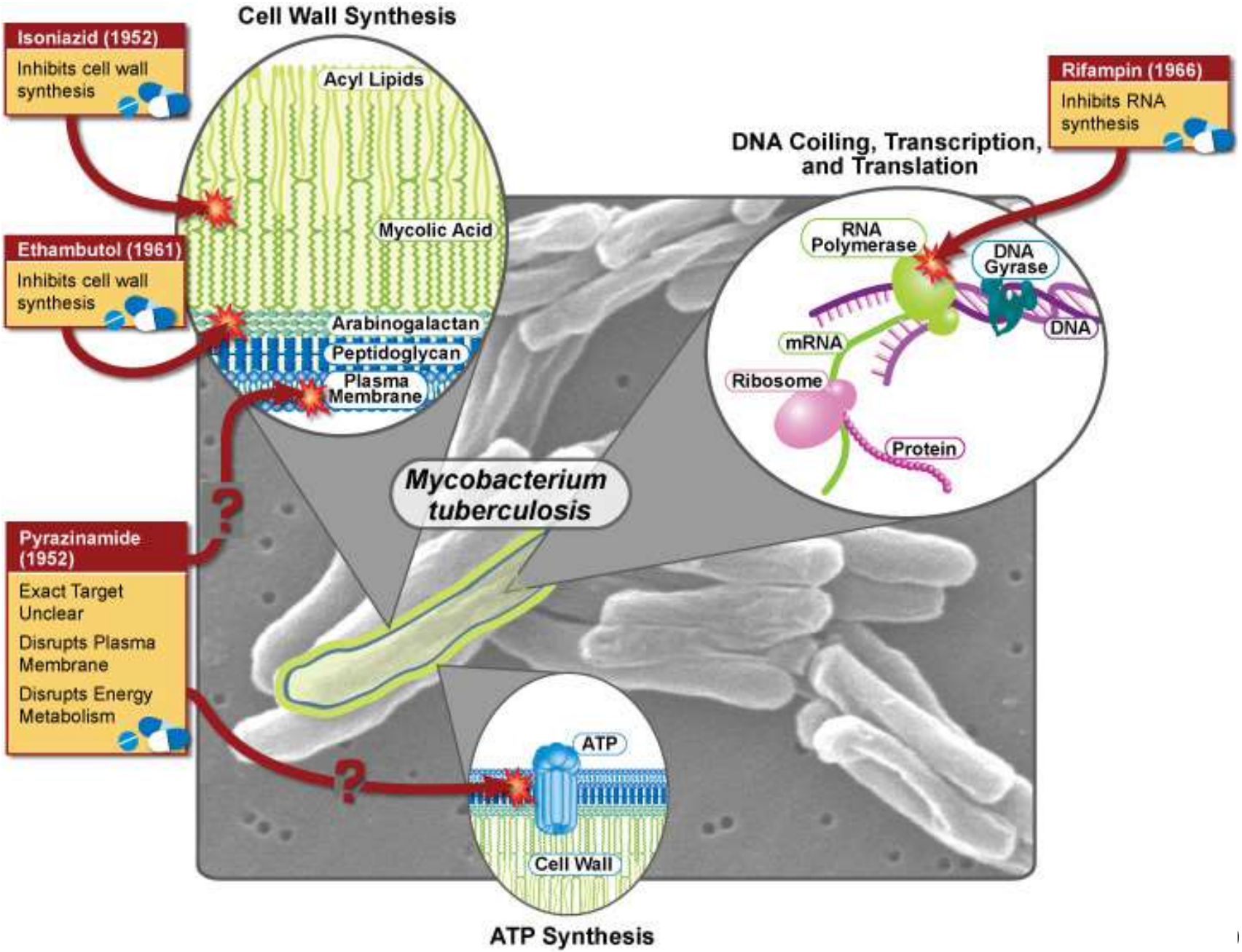
- Combination of active drugs against different populations.
- Use of drugs for prolonged periods of time

BASIS OF THE TREATMENT

Combination of three or more antituberculous drugs, over a prolonged period.

Rifampicin , isoniazid and others drugs.

http://movies-griffithbuckminsterrandall.blogspot.com/2011/03/rifampicina.html
http://laboratoriocbtis253.blogspot.com/2010/04/tratamiento-mycrobacterium-tuberculosis.html

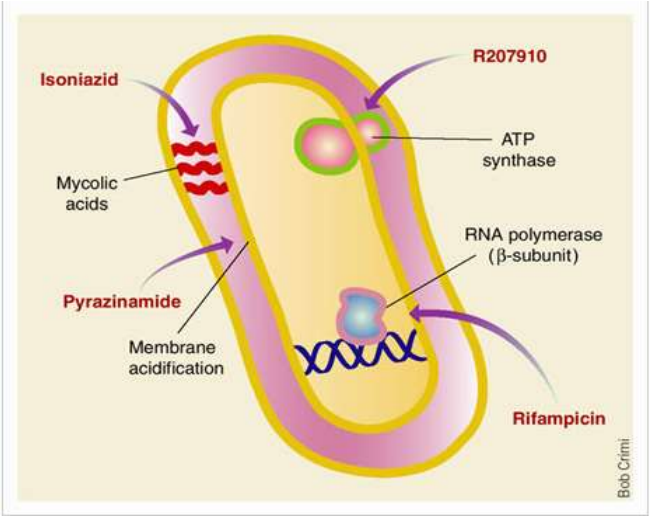

Cell Wall Synthesis
Isoniazid (1952)
Inhibits cell wall synthesis
Acyl Lipids
Mycolic Acid
Ethambutol (1961)
Inhibits cell wall synthesis
Arabinogalactan
Peptidoglycan
Plasma Membrane
Rifampin (1966)
Inhibits RNA synthesis
DNA Coiling, Transcription, and Translation
RNA Polymerase
DNA Gyrase
DNA
mRNA
Ribosome
Protein
Mycobacterium tuberculosis
Pyrazinamide (1952)
Exact Target Unclear
Disrupts Plasma Membrane
Disrupts Energy Metabolism
ATP
Cell Wall
ATP Synthesis

Treatment

- **Standard :**
  - 2 MONTHS →isoniasid, rifampicin and piracinamid,
  - 4 MONTHS → isoniasid, rifampicin
  - Immigrants from developing countries → ETHAMBUTOL is added during the first phase.

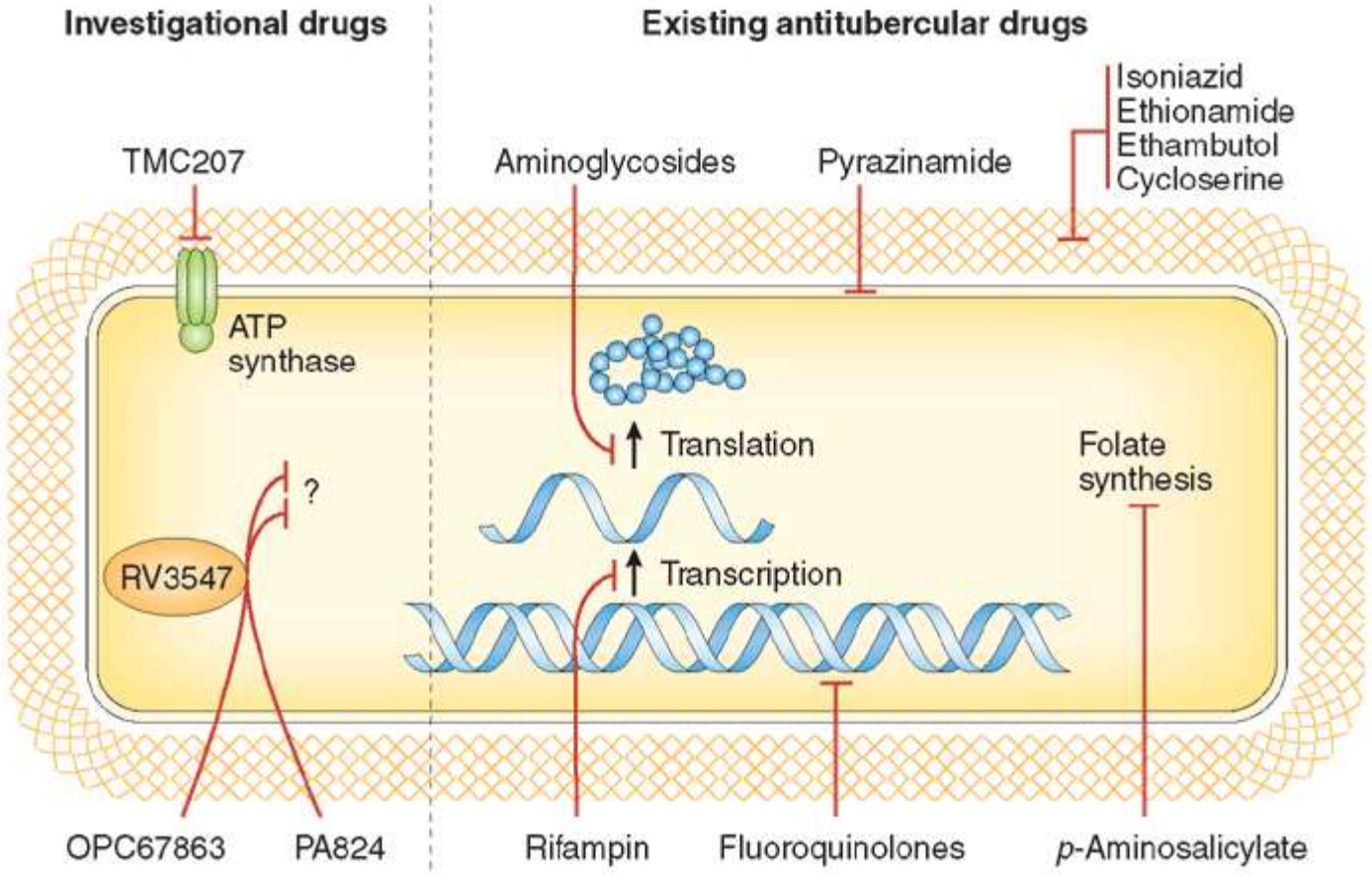
Tuberculosis therapy

- Treatment of drug-susceptible tuberculosis
  - Initial phase of isoniazid, a rifampicin, pyrazinamide and ethambutol for the first 2 months
  - Continuation phase of isoniazid and a rifampicin for the last 4 months
  - 95 % of people with DS-TB can be cured
- MDR-TB - resistance to at least isoniazid and rifampicin, first-line drugs used in the treatment of TB
  - MDR-TB is treated by a combination of eight to ten drugs with therapies lasting up to 18–24 months
  - 50 % to 70 % cured



Mitchison D.A. *et al*.: *Nature Biotechnology* 23 (2005).

Drugs againts tuberculosis

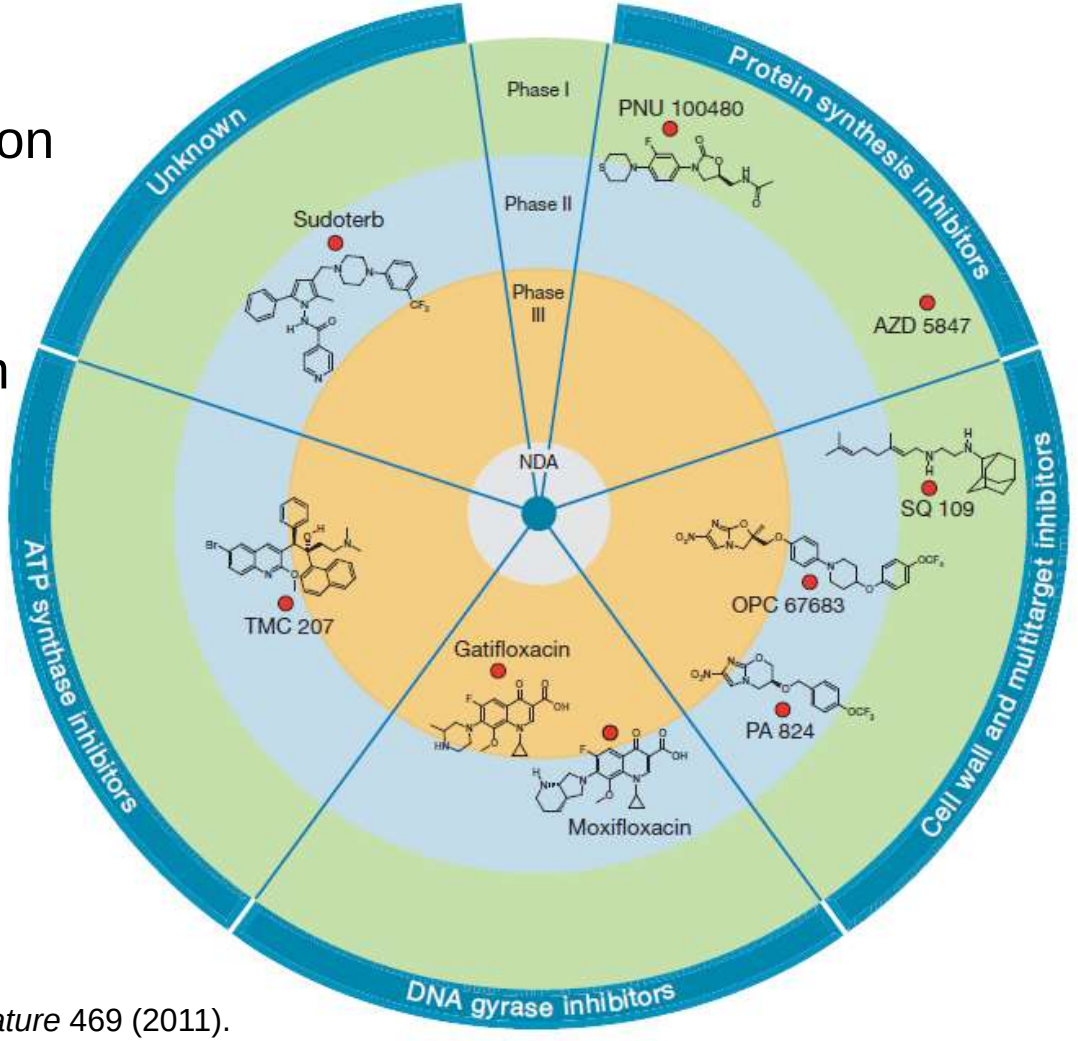


http://www.nature.com/nm/journal/v13/n3/images/nm0307-279-F1.gif

Tuberculosis therapy

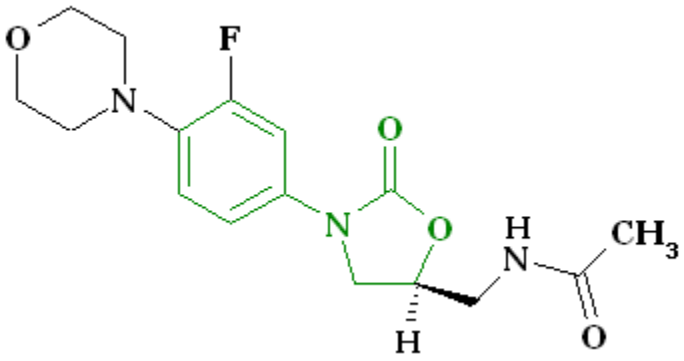
New drug discovery for tuberculosis

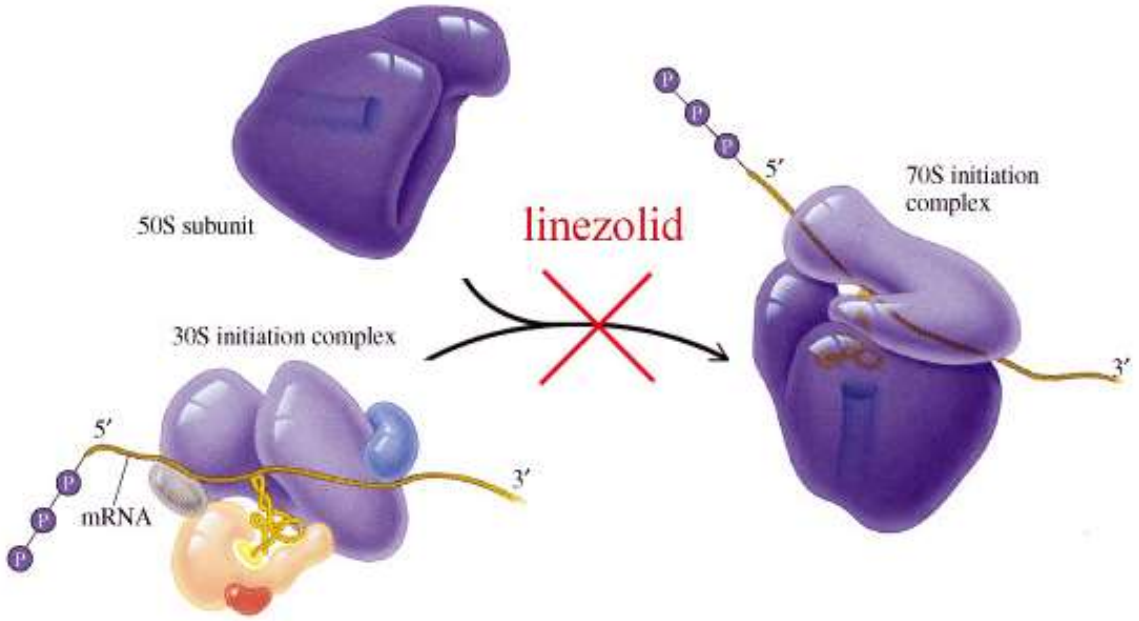
- New drug application (NDA)
- Focused on:
  - Protein sythesis inhibition
  - Cell wall inhibition
  - DNA gyrase inhibition
  - ATP synthase inhibition



Koul A. *et al*.: *Nature* 469 (2011).

New hope for Multiresistant tuberculosis treatment



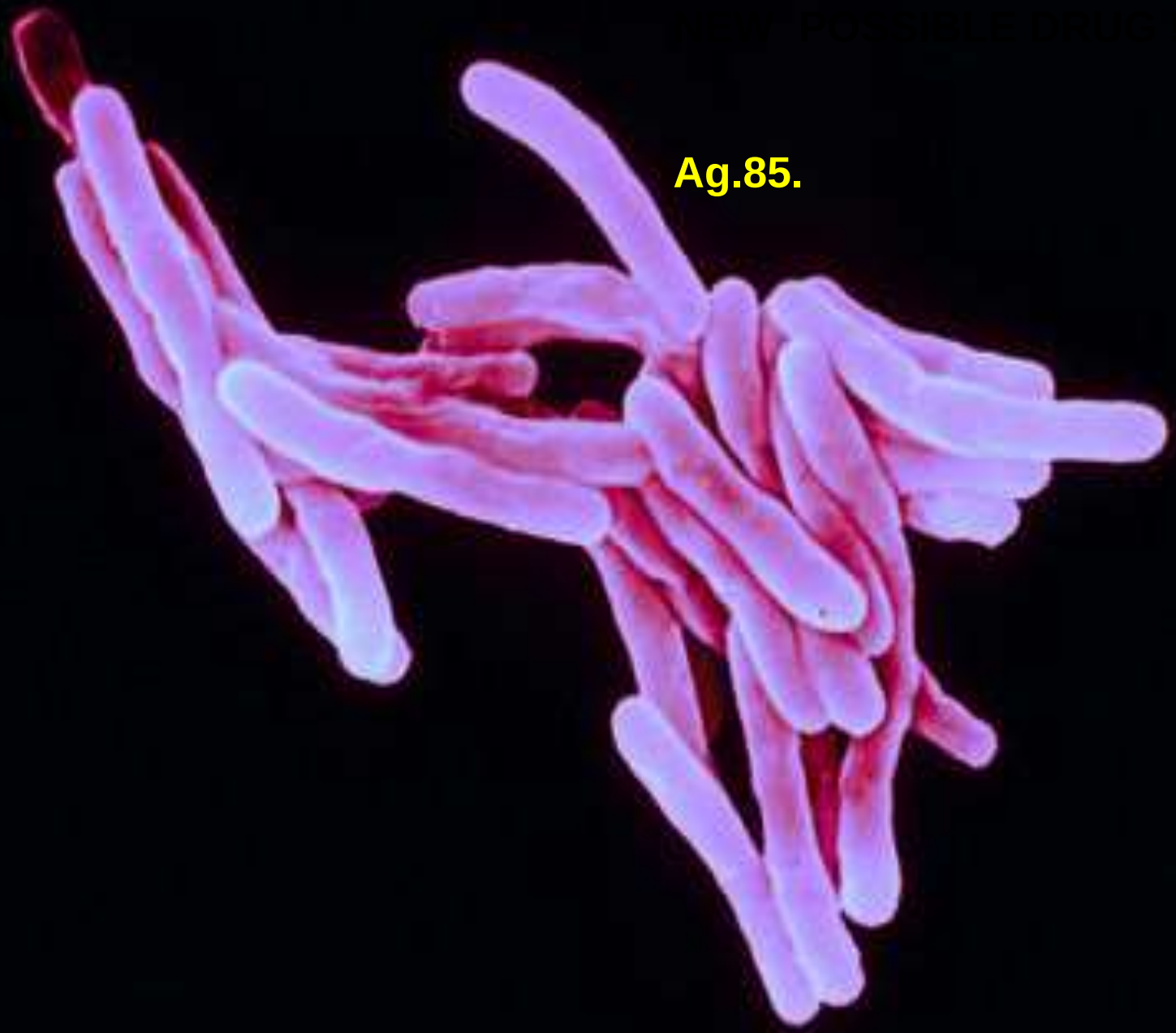


Clifton E. Barry III, Ph.D.
Chief, Tuberculosis
Research Section, NIH

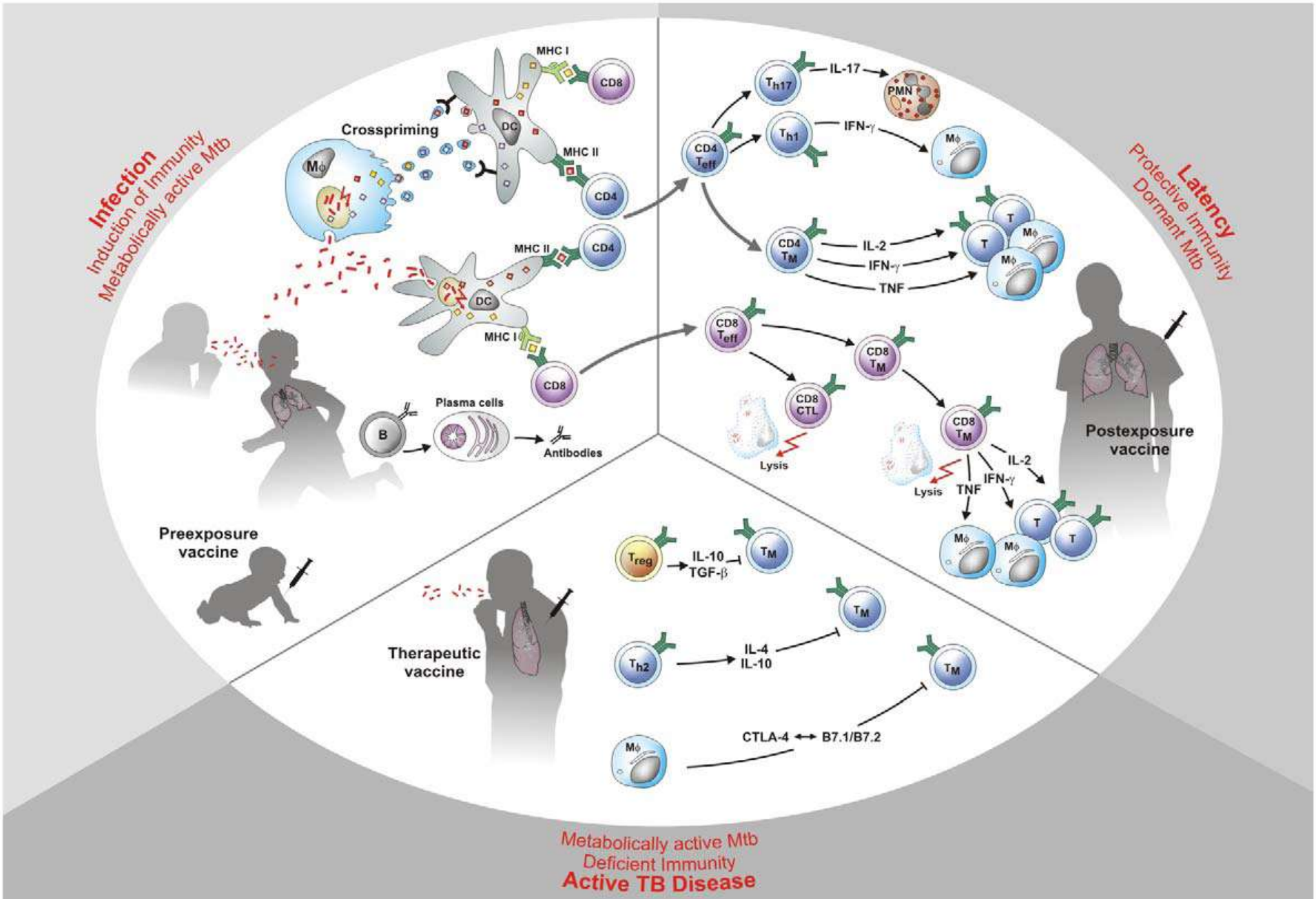
N Engl J Med. 2012 Oct 18;367(16):1508-18. doi: 10.1056/NEJMoa1201964.

Linezolid for treatment of chronic extensively drug-resistant tuberculosis.

Lee M,…. Barry CE 3rd. NIH and International Tuberculosis Research Center, Changwon, South Korea.


Ag.85.

The complexicty of M. tuberculosis infections



VACCINES

- **BCG (BACIL OF CALMETTE-GUERIN)**

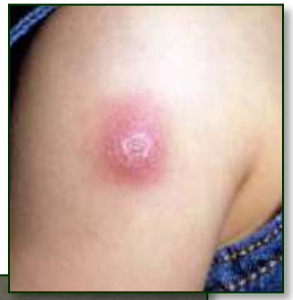
Formed by attenuated live Bacillus from a strain of Mycobacterium bovis.

SYSTEMATIC USE:

- In developing countries with high rates of infection.
- Children not infected in areas with annual risk of acquiring the infection.

SECONDARY EFFECTS:

- 2-6 WEEKS → papule is ulcer and forms a crust.
- Cure in 8-12 weeks, leaving a scar



Papule

CONTRAINDICATIONS:

- People with immunodeficiency(VIH)
- Individuos con infección TB previa
- Pregnancy

Vacine BCG.

http://www.lung.ca/tb/images/full_archive/149_bcg_vaccine.jpg; http://www.pediatria24.com/wp-content/uploads/bcg.jpg

BUT

- People vaccined with BCG→+ve skin testing.

- During the adulthood, does not protect to reactivation of latent infection, rapid progression of primary infection, or re-infection.

- The efficacy of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccine against pulmonary tuberculosis (TB) varies enormously in different populations.

SO

- Reintroducing into *M. microti* the complete region of difference 1 (RD1).

M. microti OV254::RD1-2F9

- **Induced specific immune responses in mice with CD8+ T lymphocytes that had strong expression of the CD44^{hl}activation marker.**

- **A good target gene to replace is the Nramp1 which messure the susceptibility of a person.**
- In fact, it has been shown that the DC of healthy individuals produce IFN-γ in response to stimulation with BCG a TLR2-dependent mechanism .

Most advanced TB vaccine candidates in clinical trials

Type	Candidate	Description	Clinical Trial Status
Recombinant BCG for pre-exposure prime vaccination	VPM 1002	rBCG-expressing listeriolysin and urease deletion	Phase IIa ongoing
	rBCG30	rBCG-expressing Ag85B	Phase I completed/on hold
	Aeras-422	rBCG-expressing perfringolysin and Ag85A, 85B, Rv3407	Phase I terminated due to side effects
Viral-vector for pre-exposure booster vaccination	Oxford MVA85A/Aeras-485	Modified vaccinia Ankara-expressing Ag85A	Phase IIb ongoing
	Crucell Ad35/Aeras-402	Replication-deficient adenovirus 35-expressing Ag85A, Ag85B, TB10.4	Phase IIb ongoing
	AdAg85A	Replication-deficient adenovirus 5-expressing Ag85A	Phase I
Fusion protein in adjuvant for pre-exposure booster vaccination	Hybrid 1+IC31	Fusion of Ag85B and ESAT-6 in adjuvant IC31	Phase I, soon entering IIa
	Hybrid 56+IC31	Fusion of Ag85B, ESAT-6 and Rv2660c in adjuvant IC31	Phase I ongoing
	Hybrid 1+CAF01	Fusion of Ag85B and ESAT-6 in adjuvant CAF01	Phase I ongoing
	M72+AS01 or AS02	Fusion of Rv1196 and Rv0125 in adjuvant AS01 or ASO2	Phase IIa ongoing
	Aeras-404: HyVac4+IC31	Fusion of Ag85B and TB10.4 in adjuvant IC31	Phase I
Whole bacterial vaccine for therapeutic vaccination	RUTI	Detoxified *M. tuberculosis* in liposomes	Phase IIa ongoing
	M. vaccae	Inactivated *M. vaccae*	Phase III completed

References

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Thank you for attention