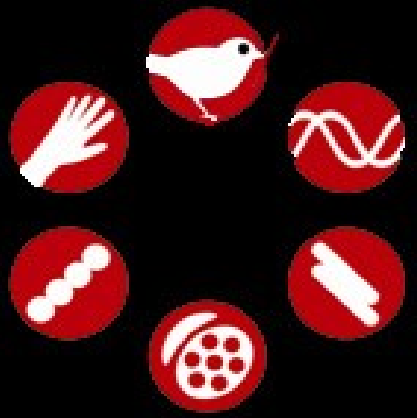
Steffen Stenger
Medical Microbiology and Hygiene
University Hospital Ulm
MyTB Lab



Fight against Tuberculosis: General Aspects

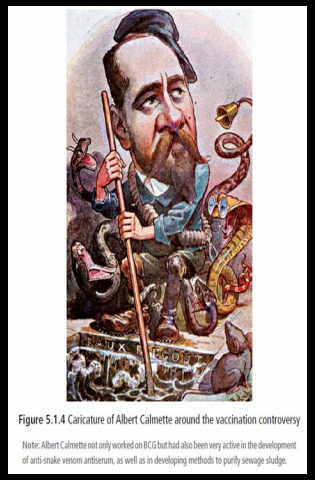
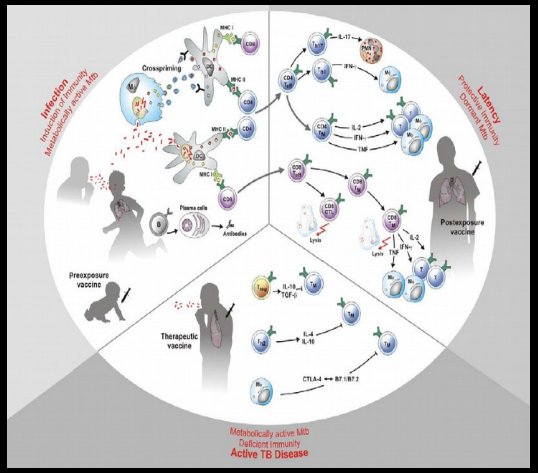
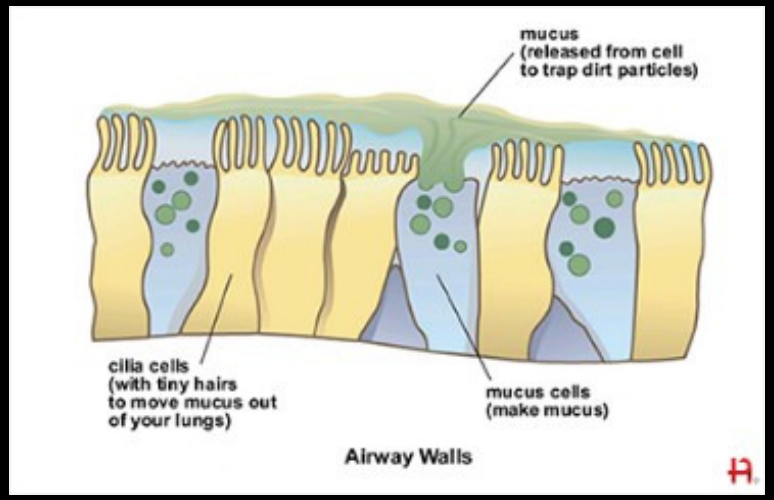


Figure 5.1.4 Caricature of Albert Calmette around the vaccination controversy

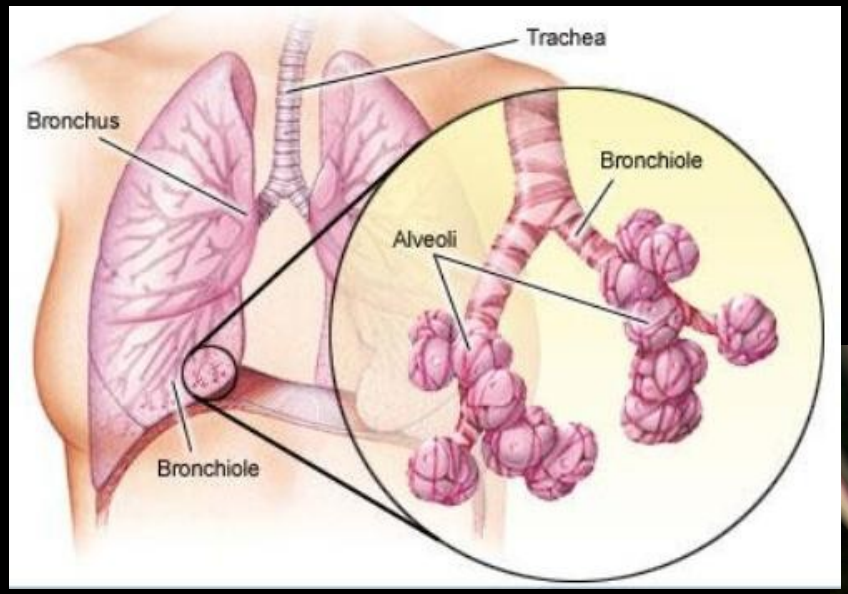
Note: Albert Calmette not only worked on BCG but had also been very active in the development of anti-snake venom antiserum, as well as in developing methods to purify sewage sludge.

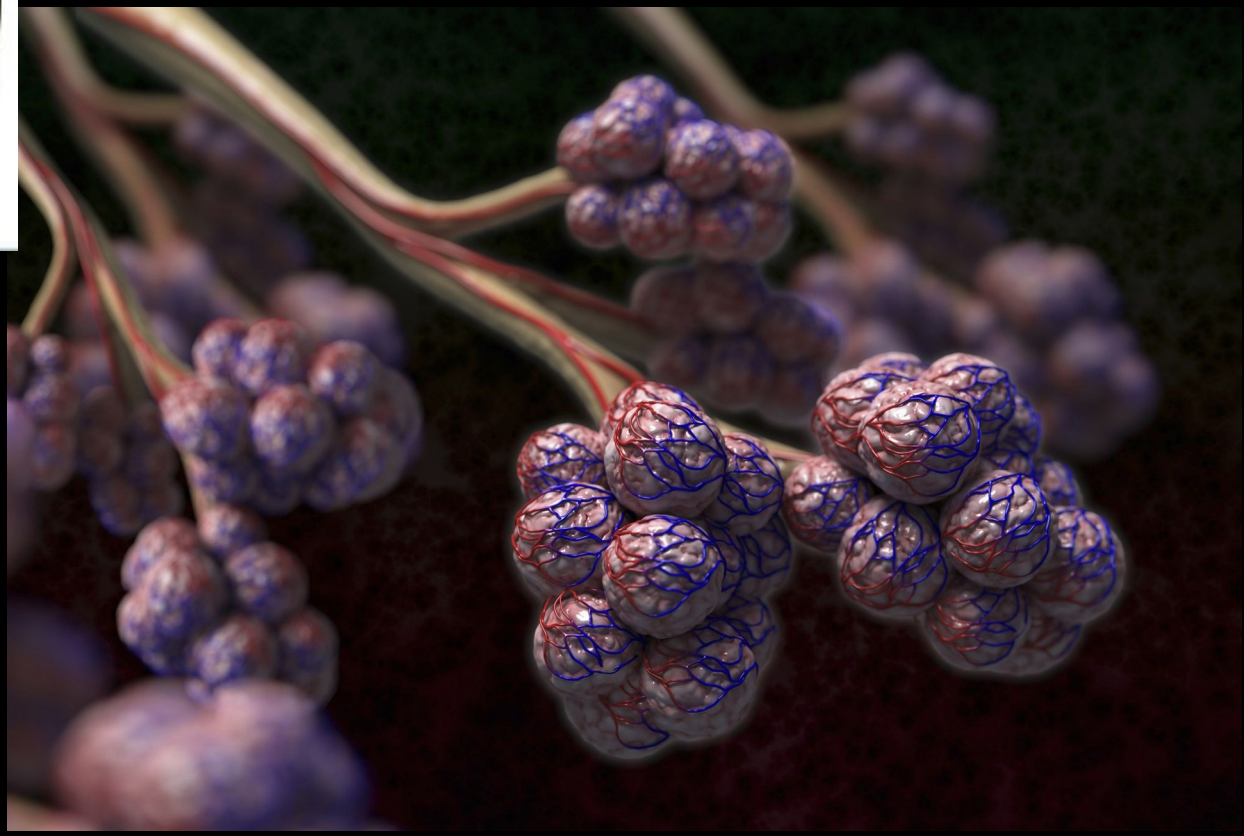


Physical barrier of the respiratory epithelium

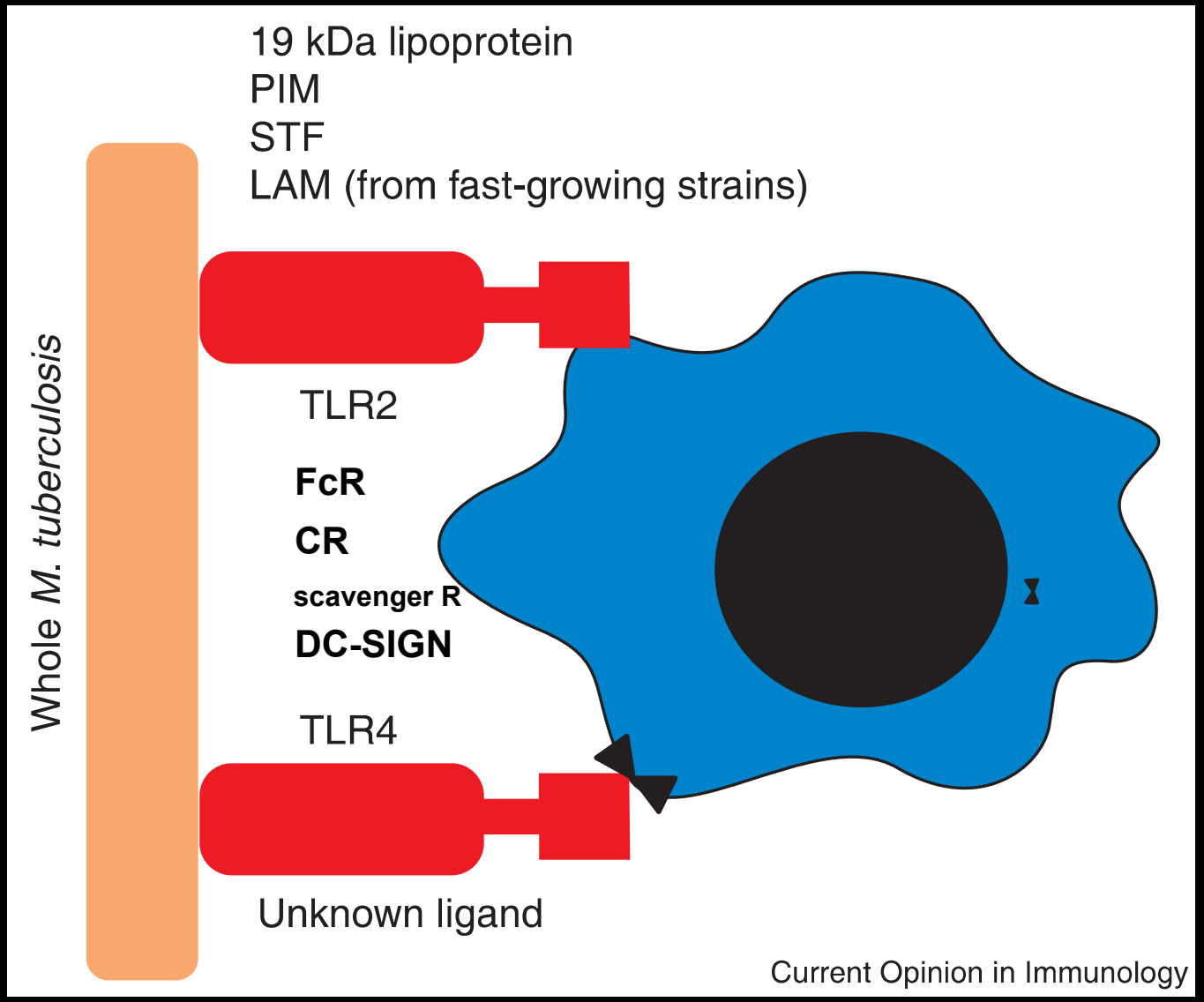


Mycobacteria (may) pass the upper airways and reach the alveolar space



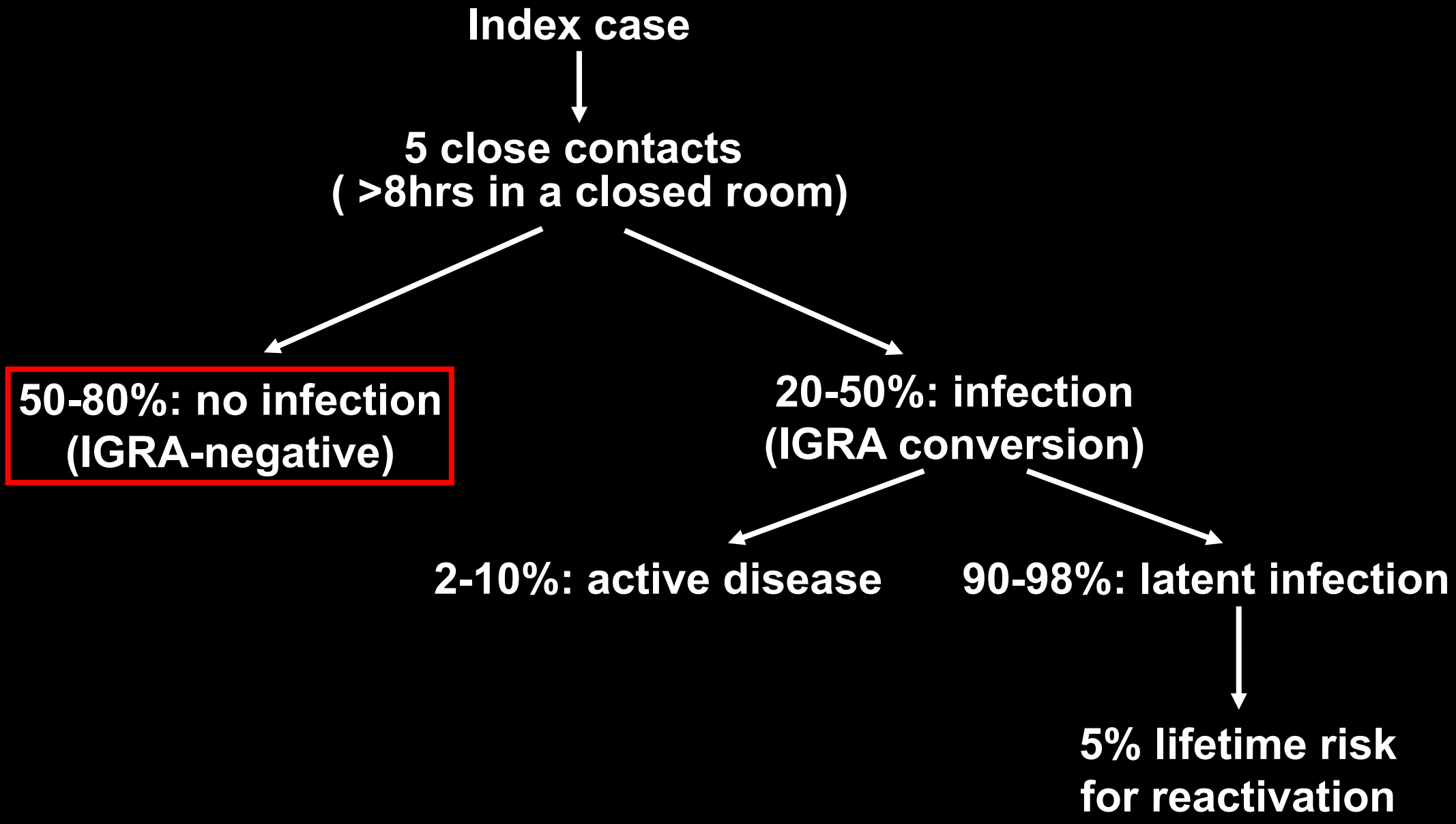


.....where they interact with pattern recognition receptors of macrophages and dendritic cells

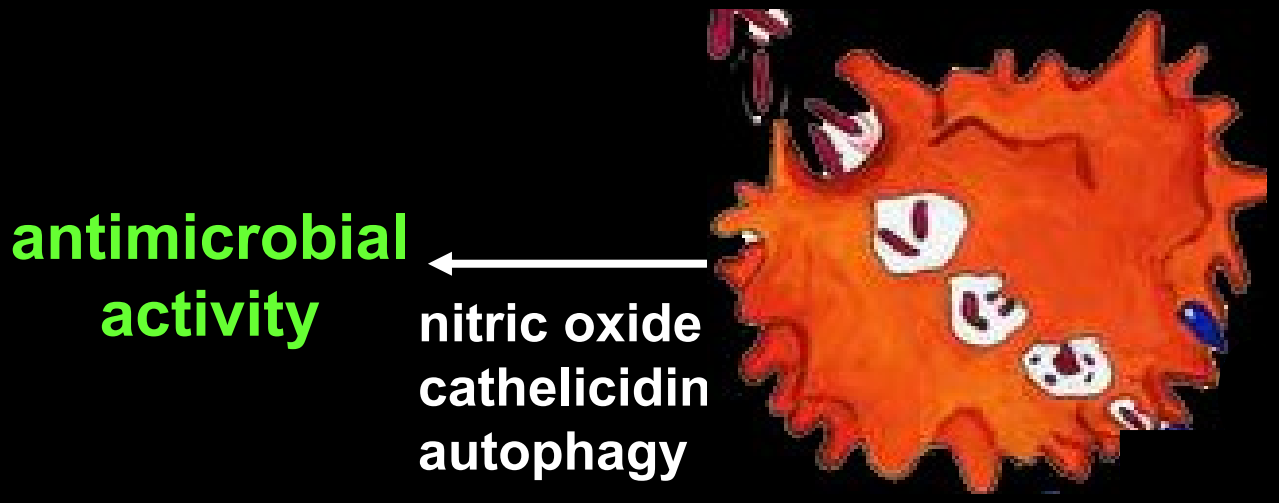


Stenger et al. , Curr. Opin. Immunol., 14: 452, 2002

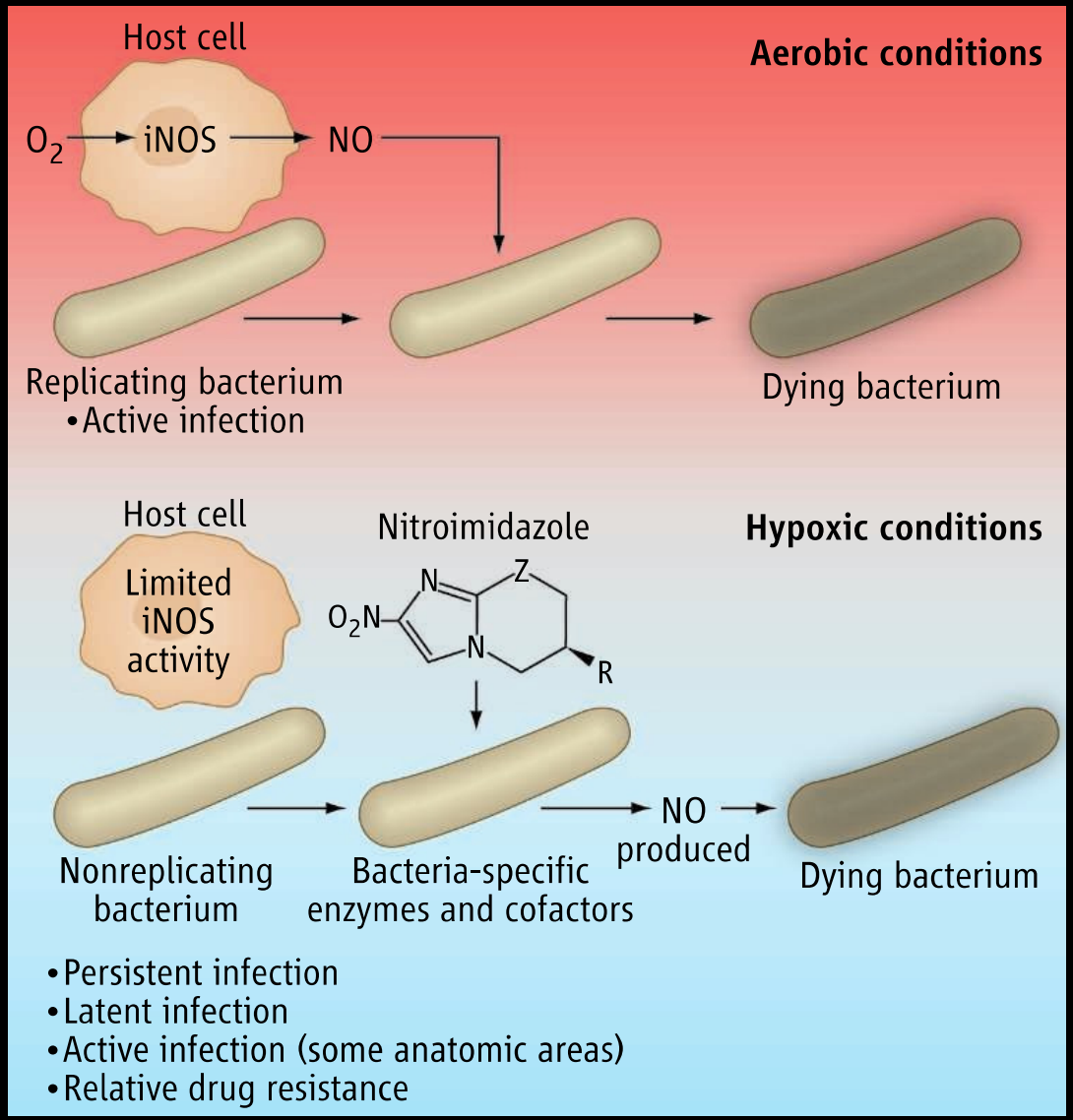
The physical barrier of the upper airways and innate immune cells eliminate the majority of bacilli



Immunity to *Mycobacterium tuberculosis*



PA-824 kills nonreplicating *Mycobacterium tuberculosis* by intracellular NO-release: An antibiotic mimics immunity



Singh, Science, 322: 1392, 2008
Nathan, Science, 322: 1337, 2008

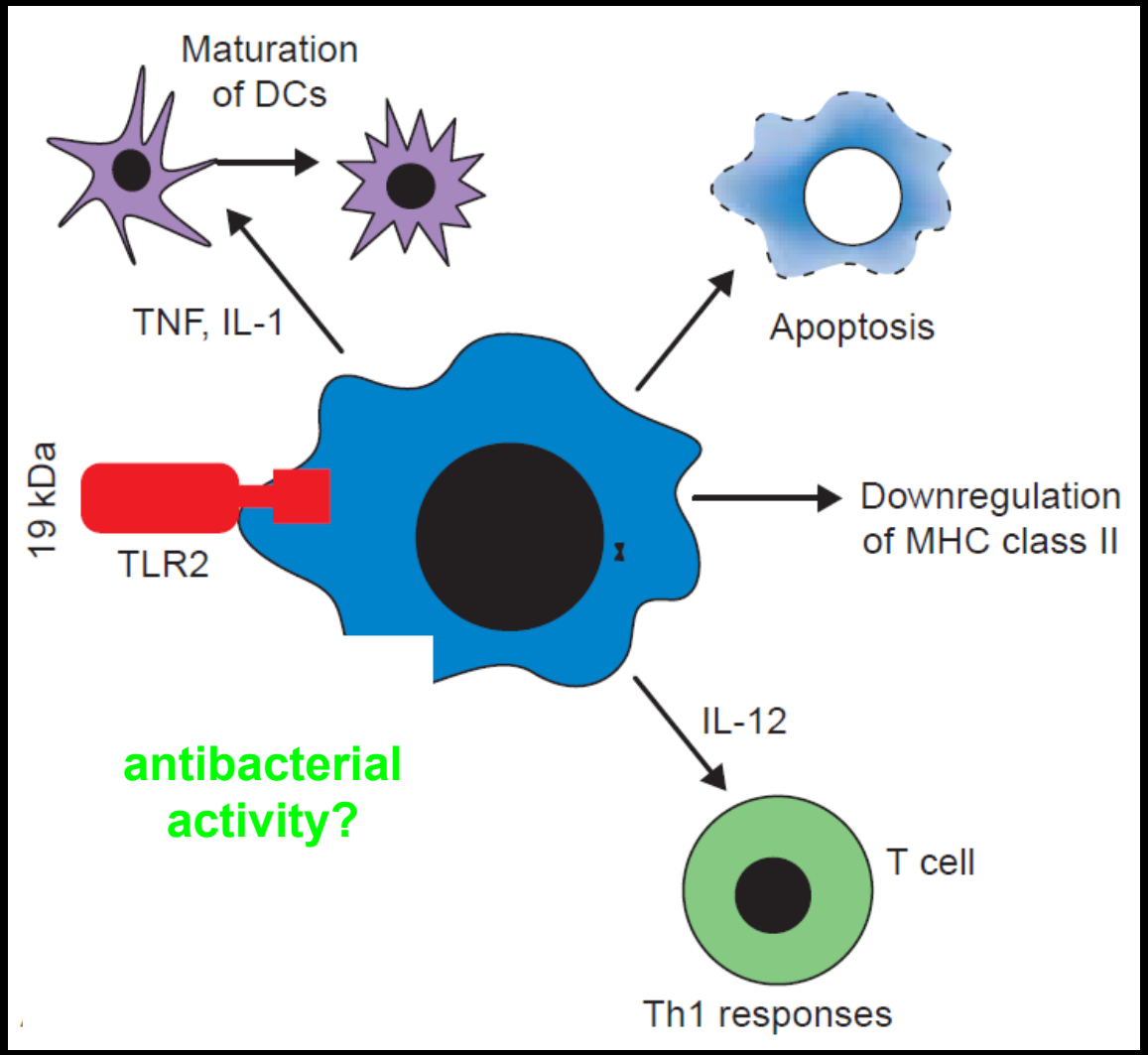
Pretonamid (PA-824) as a therapy against tuberculosis

Drug	Class	Sponsor(s)	Phase

http://www.tbonline.info/medicines/

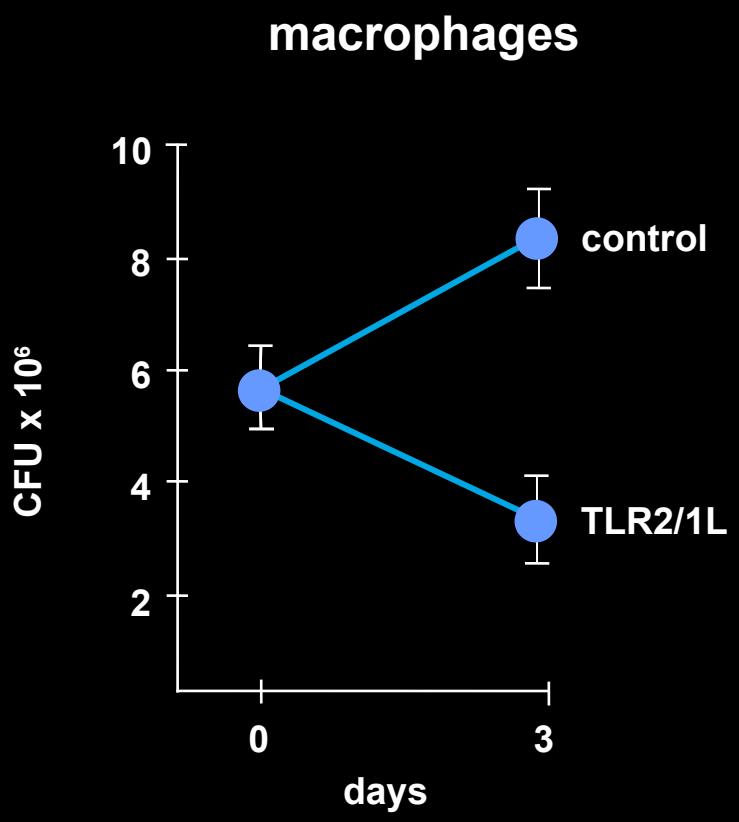
- QT-prolongation, liver toxicity (3 fatalities)

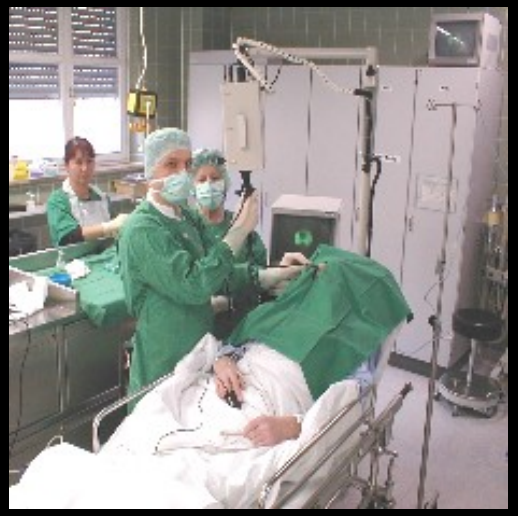
Toll like receptors sense *Mycobacterium tuberculosis* and induce innate and acquired immunity

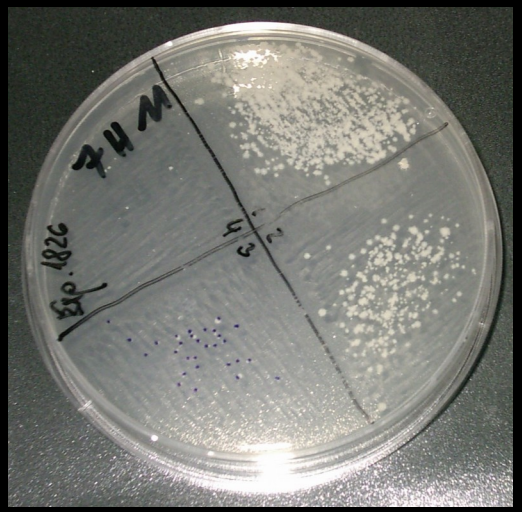


Stenger et al. , Curr. Opin. Immunol., 14: 452, 2002

TLR2/1 ligands induce antimicrobial activity in macrophages

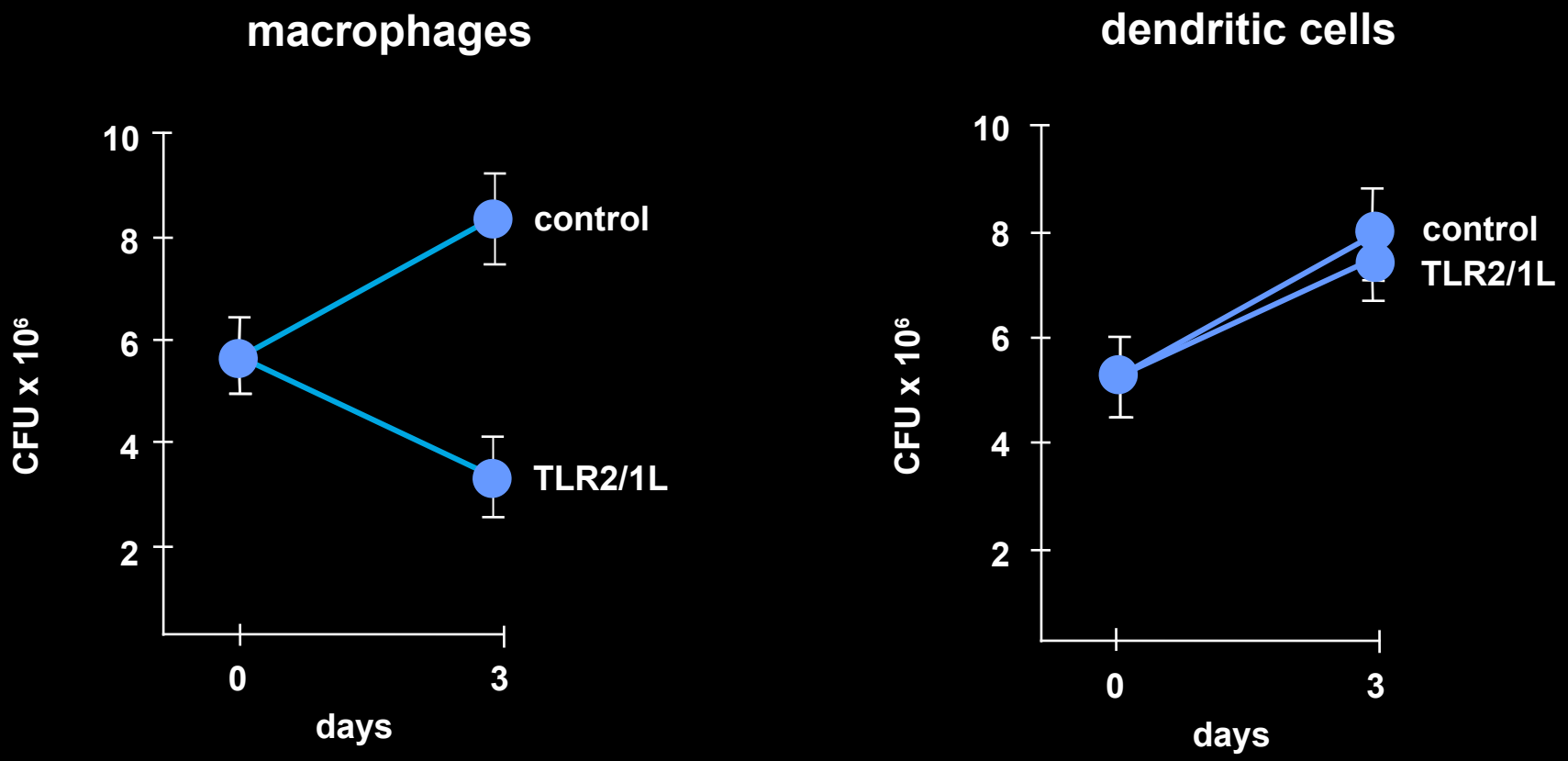






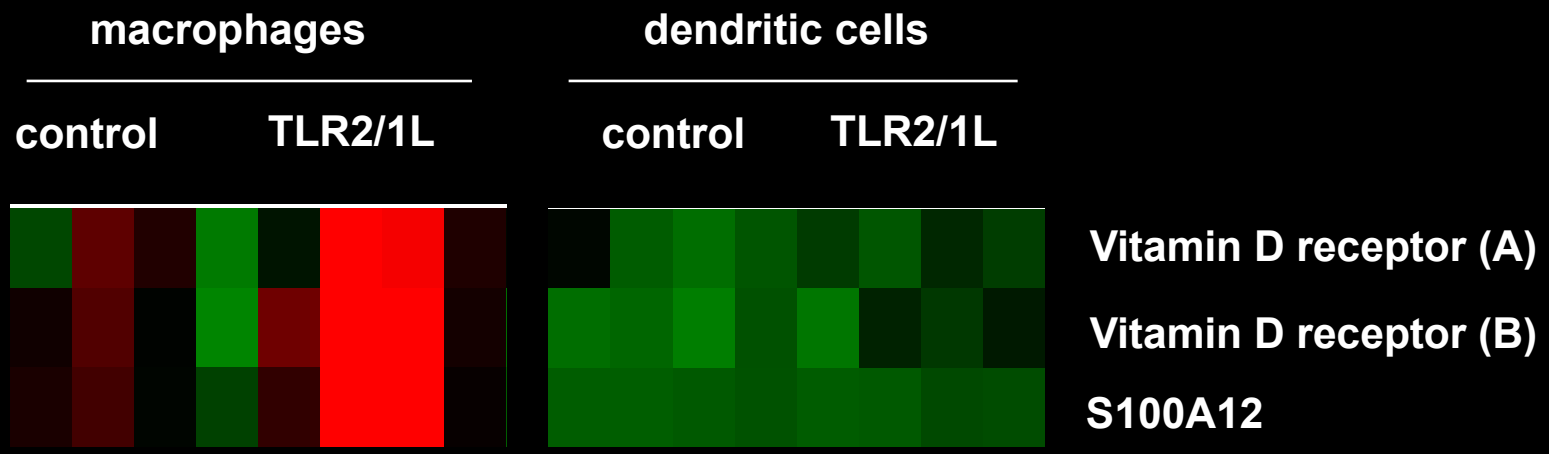
Liu et. al., Science, 291, 2001

TLR2/1 ligands induce antimicrobial activity in macrophages, but not dendritic cells



BAD NEWS CAN BE GOOD NEWS (FOR SMART SCIENTISTS)

Search for immunomodulatory genes that are selectively induced in macrophages



Vitamin D and tuberculosis

VDR polymorphisms m… …ycobacterial disease
(Bellamy et al., J Infect Dis, 179: 721, 19… …j, J Clin. Immunol, 24: 523, 2004;
Bornmann et al, J. Infect. Dis., 190: 163…

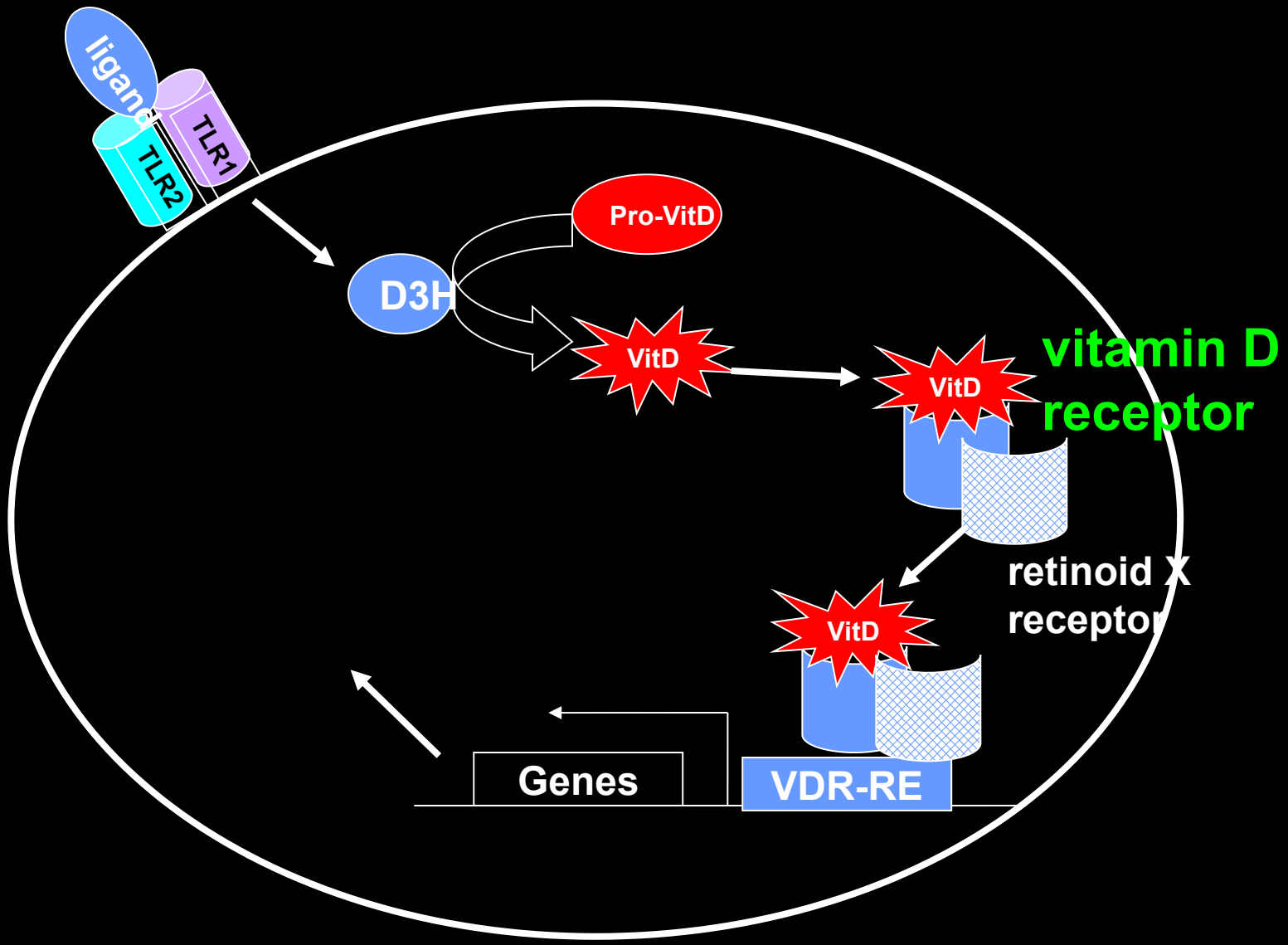
Correlation between low… …the risk
to develop tuberculosis… …, Lancet, 355: 618, 2000)

Tuberculosis patients h… …n healthy contacts
(Sita-Lumsden et al., Thorax, May 2007)

Vit D3 induces the **oxida**… …**lysosome fusion**
in *M.Tb*-infected macrop… …01; Hmama, J. Cell Sci, 117:
2131, 2004)

Vit D3 enhances **antimy**… …es and
macrophages (Rook et al., Imm… …., 55: 2945, 1987; Denis et
al., Clin Exp. Imm., 84: 200, 1991; Rocke…

Hypothesis


ligand
TLR1
TLR2
Pro-VitD
D3H
VitD
VitD
vitamin D
receptor
retinoid X
receptor
VitD
Genes
VDR-RE

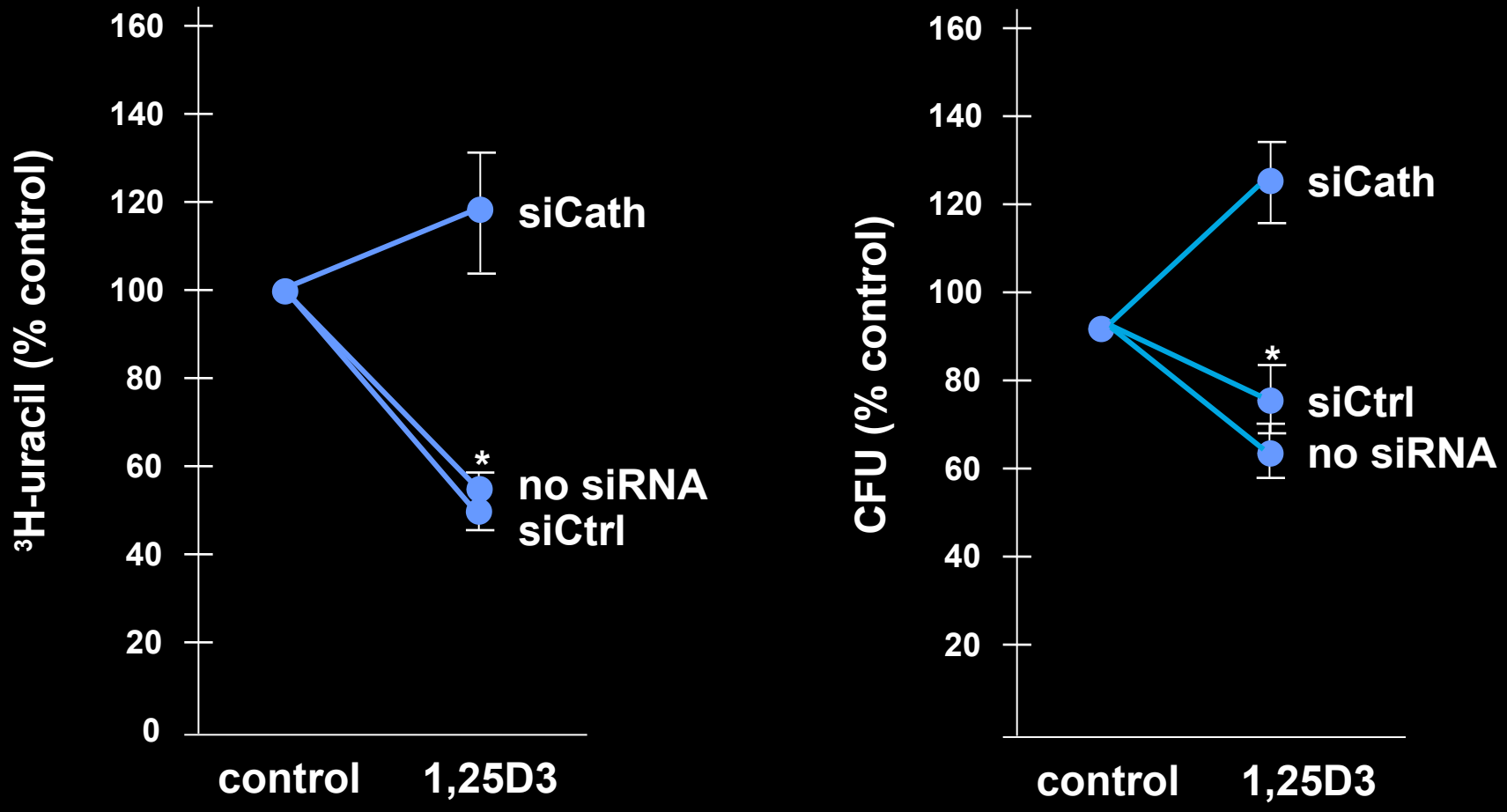
Cathelicidin

The promoter of the human cathelicidin peptide has a VitD response element (Wang et al., J. Immunol., 173: 2909, 2004)

Expression in macrophages, lymphocytes, neutrophils and epithelial cells (Wah et al., Cell Tissue Res., 324: 449, 2006)

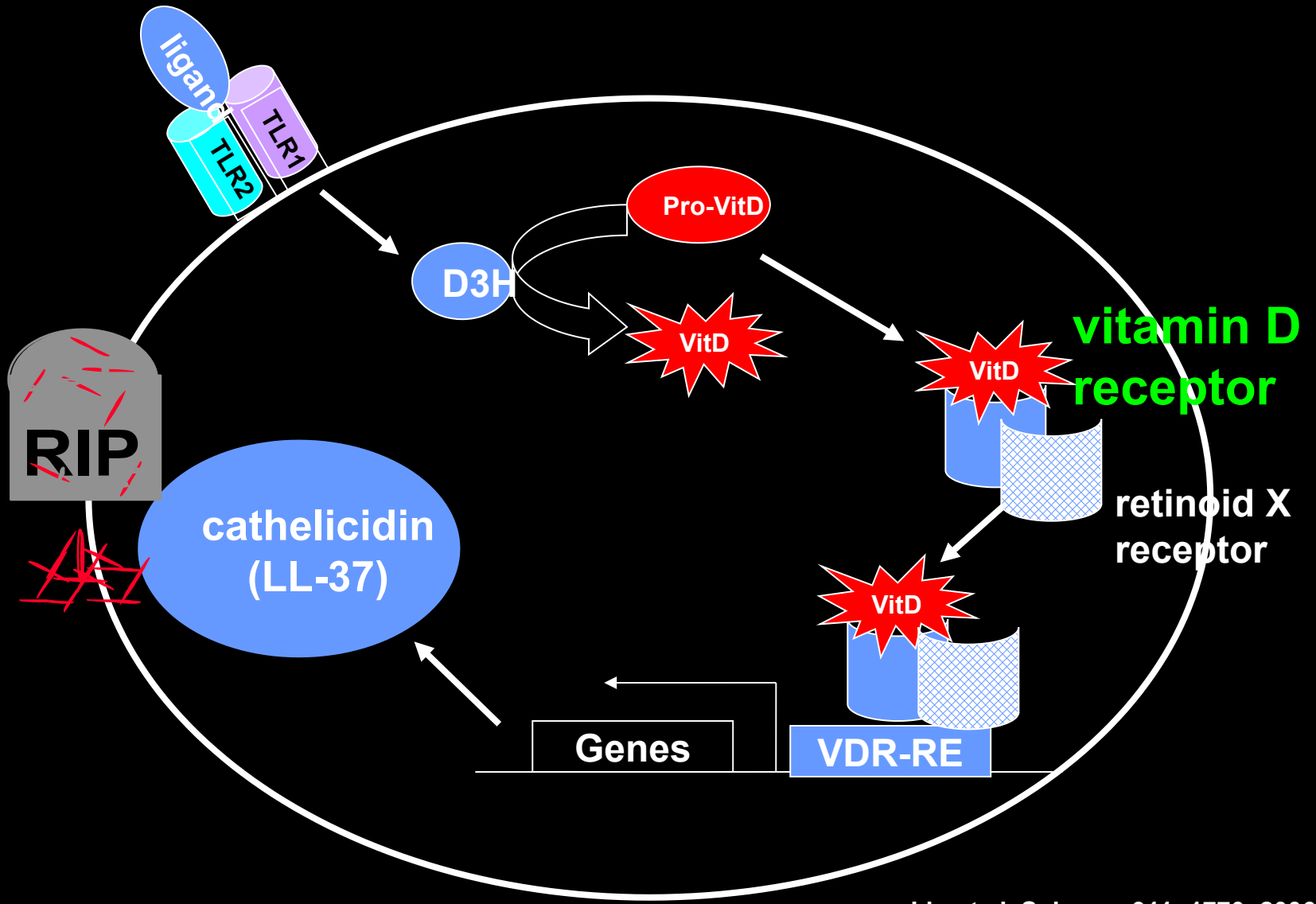
LL-37 is chemotactic, induces degranulation of mast cells, stimulates wound healing, prevents LPS-mediated sepsis in mice and kills a broad spectrum of microbes by perturbating the cell membrane (Scott, J. Immunol., 169: 3883, 2004; Niyonsaba,Eur. J. Immunol., 31: 1066, 2001; Heilborn, J. Invest Dermatol., 120: 379, 2003; Henzler-Wildmann, Biochemistry, 43: 8459, 2004)

siCath specifically knocks down VitD mediated antimicrobial activity



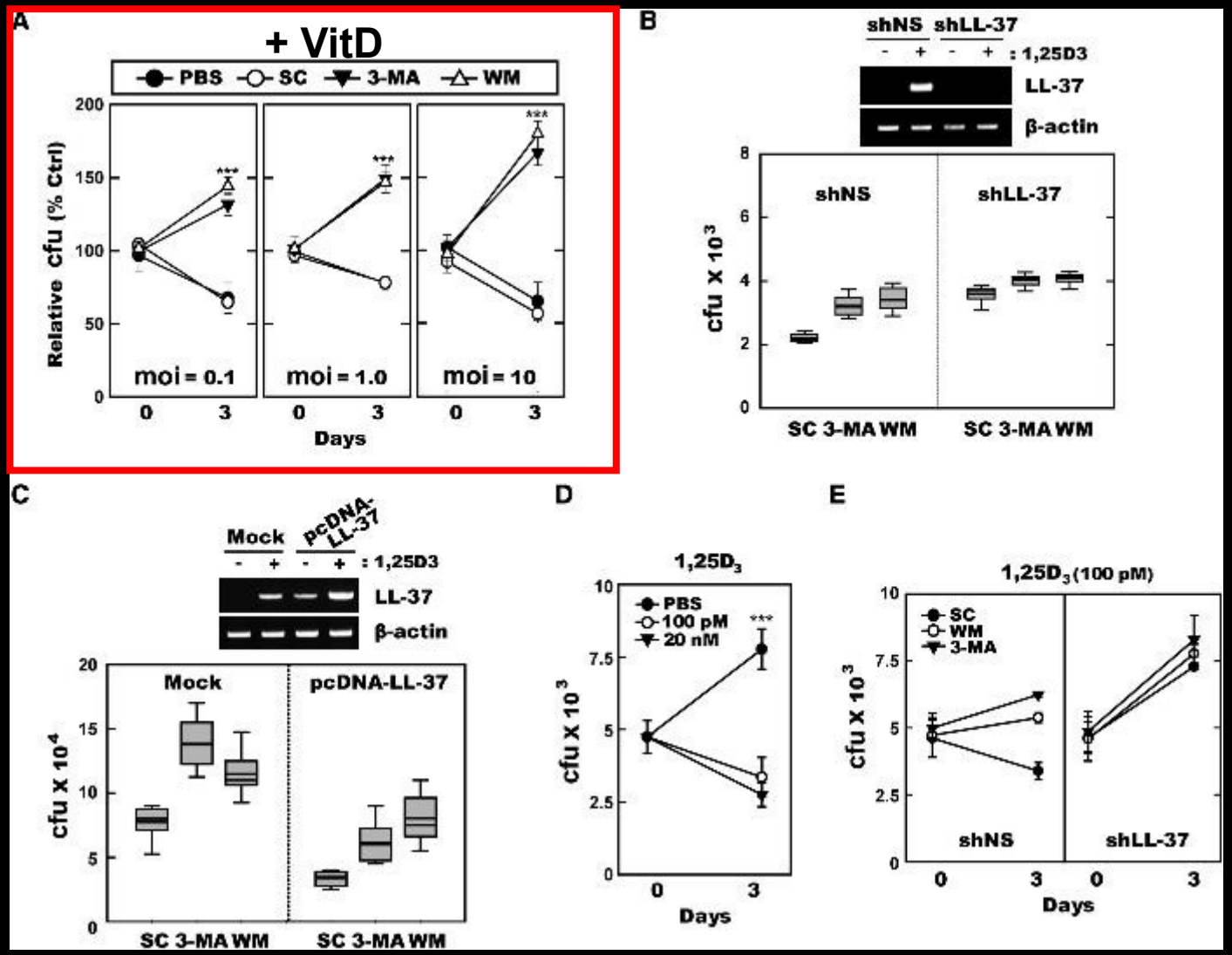
* p<0.05

Conclusion



Liu et al, Science, 311: 1770, 2006

Vitamin D-mediated antimicrobial activity is dependent on Cathelicidin and Autophagy



Yuk et al., Cell Host Microbe, 6: 231, 2009

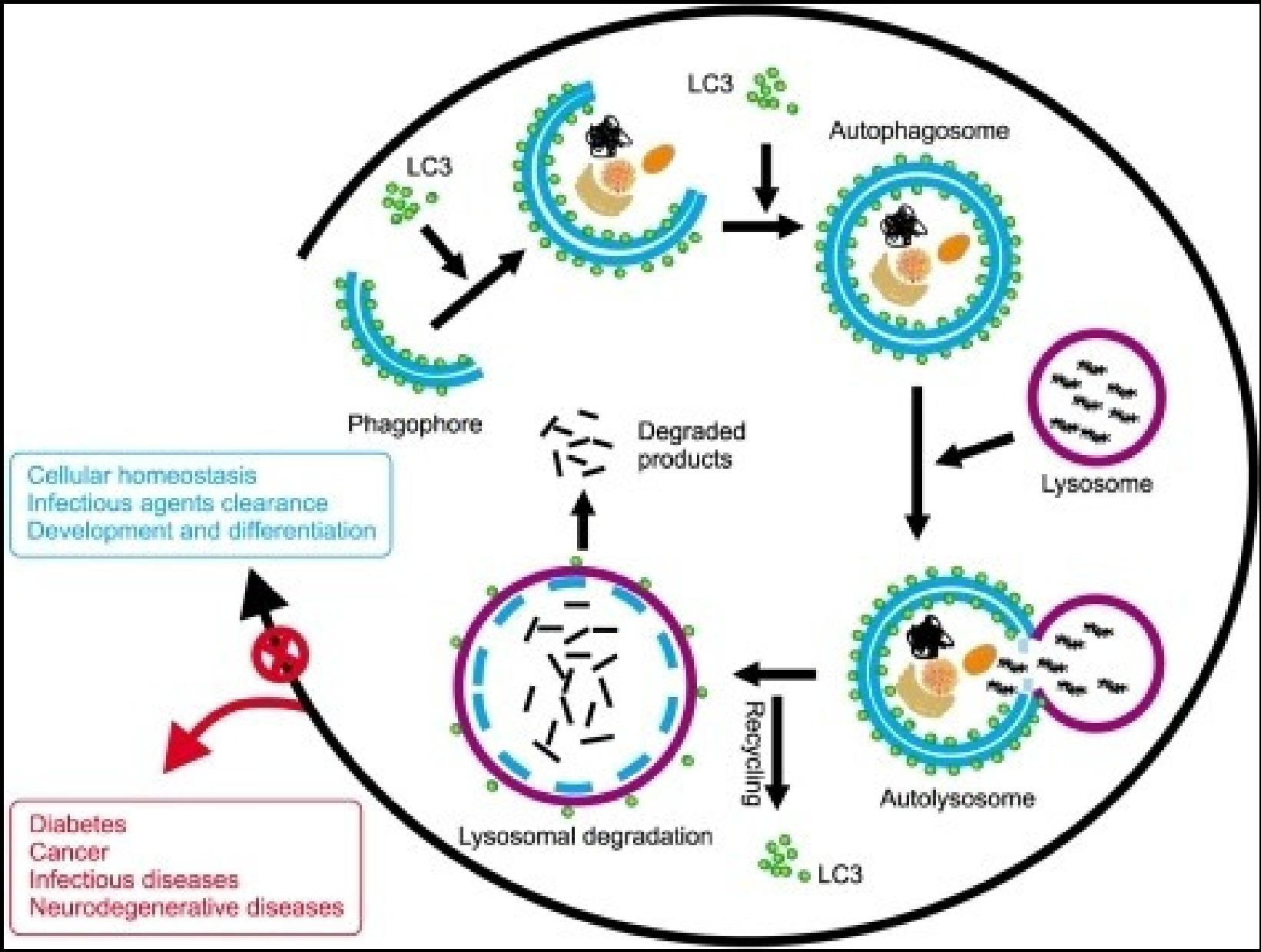
Cell, Vol. 119, 753–766, December 17, 2004, Copyright ©2004 by Cell Press

Autophagy Is a Defense Mechanism Inhibiting BCG and *Mycobacterium tuberculosis* Survival in Infected Macrophages

Maximiliano G. Gutierrez,[1,2] Sharon S. Master,[2] Sudha B. Singh,[2] Gregory A. Taylor,[3] Maria I. Colombo,[1,*] and Vojo Deretic[2,4,*]

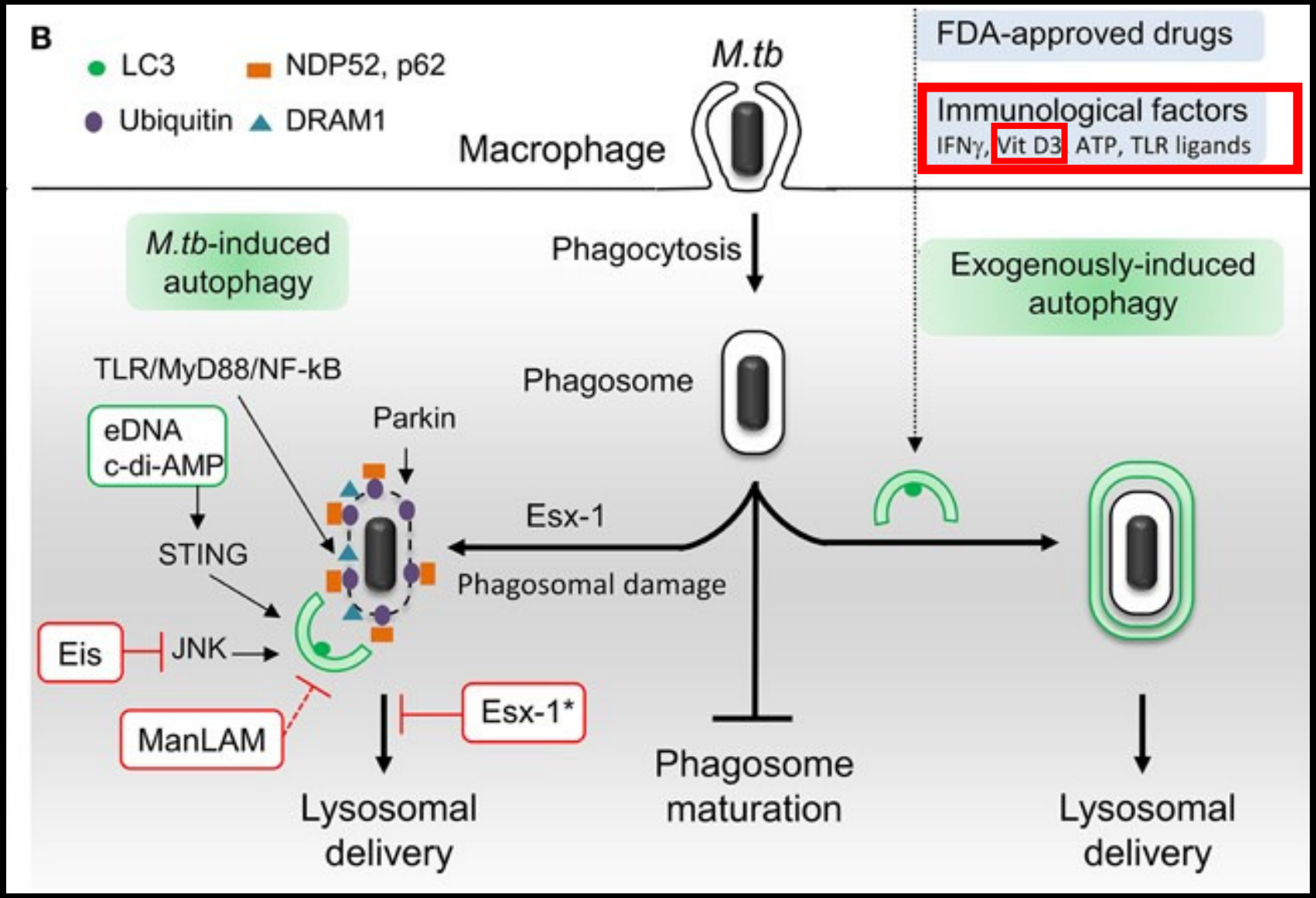
nomenon referred to in the classical
inhibition of phagosome-lysosome fu
and Hart, 1971). *M. tuberculosis* phag

Autophagy: Mechanism



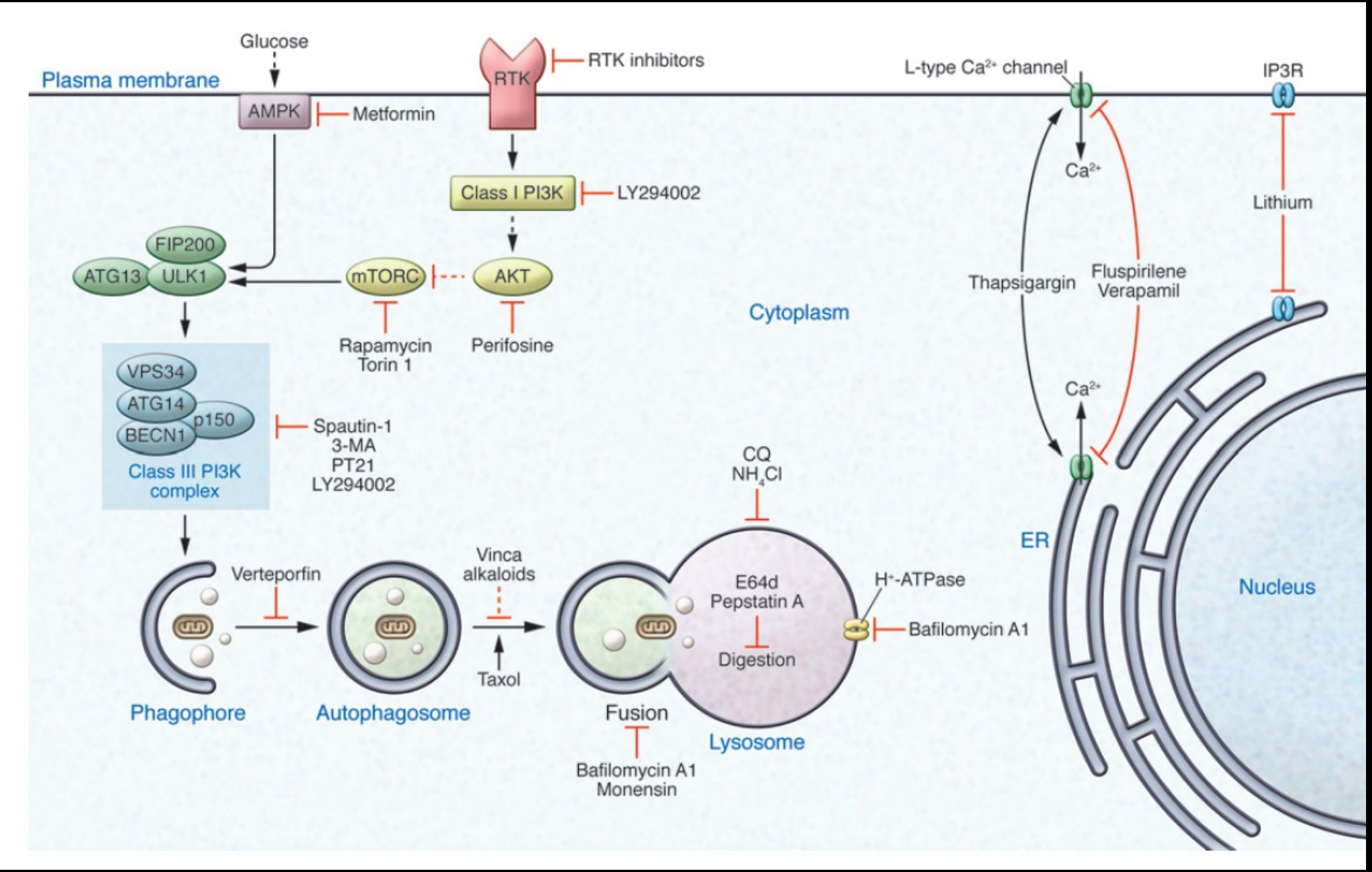
https://openi.nlm.nih.gov/imgs/512/193/3296814/3296814_emm-44-69-g002.png

Immunological Factors that induce Autophagy



Espert et al., Front Cell Infect Microbiol, 5, 2015

Pharmacological Agents that Target Autophagy



Vakifahmetoglu et al., J Clin Invest. 2015;125(1):5–13. doi:10.1172/JCI73937.

Evaluation of vitamin D as therapy against tuberculosis

Basic finding: Vit D supports antimycobacterial activity of human macrophages

Advantage: well tolerated, available as an oral drug, cheap

Next step: clinical evaluation

Vitamin D as supplementary treatment for tuberculosis

(Wejse et al., Am J Respir Crit Care Med, 179: 843, 2009)

- double blind, randomized, placebo controlled trial

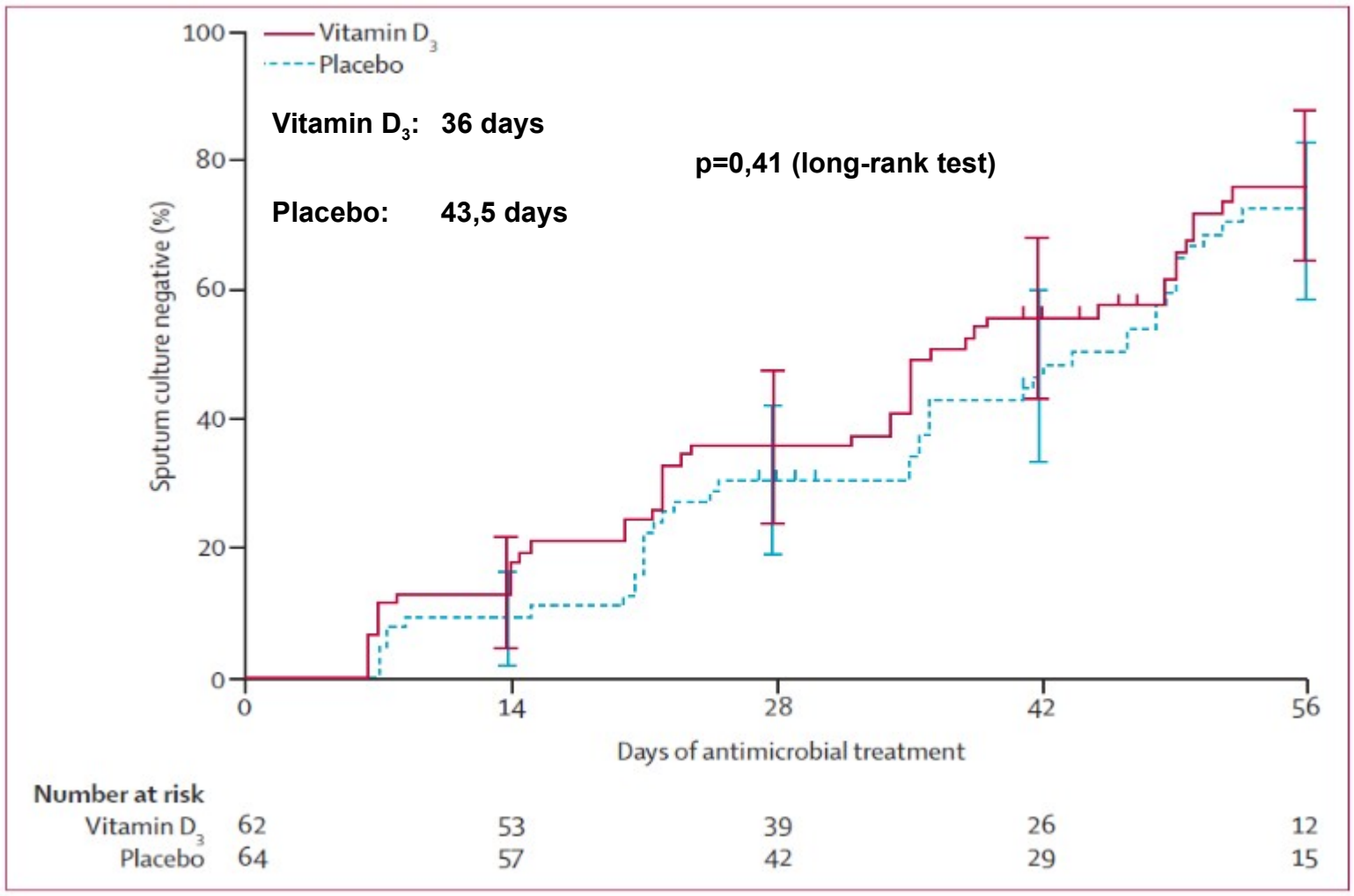
- 365 tuberculosis patients, 281 completed follow up

- Vitamin D intervention: 100.000 IU at months 0, 5, 8

- no significant differences in

  side effects

High doses of vitamin D_3 fail to reduce the time to sputum culture conversion



Martineau et al., Lancet, 377: 242, 2011

Vitamin D as supplementary treatment for tuberculosis

(Wejse et al., Am J Respir Crit Care Med, 179: 843, 2009)

- double blind, randomized, placebo controlled trial

- 365 tuberculosis patients, 281 completed follow up

- Vitamin D intervention: 100.000 IU at months 0, 5, 8

- no significant differences in

  side effects

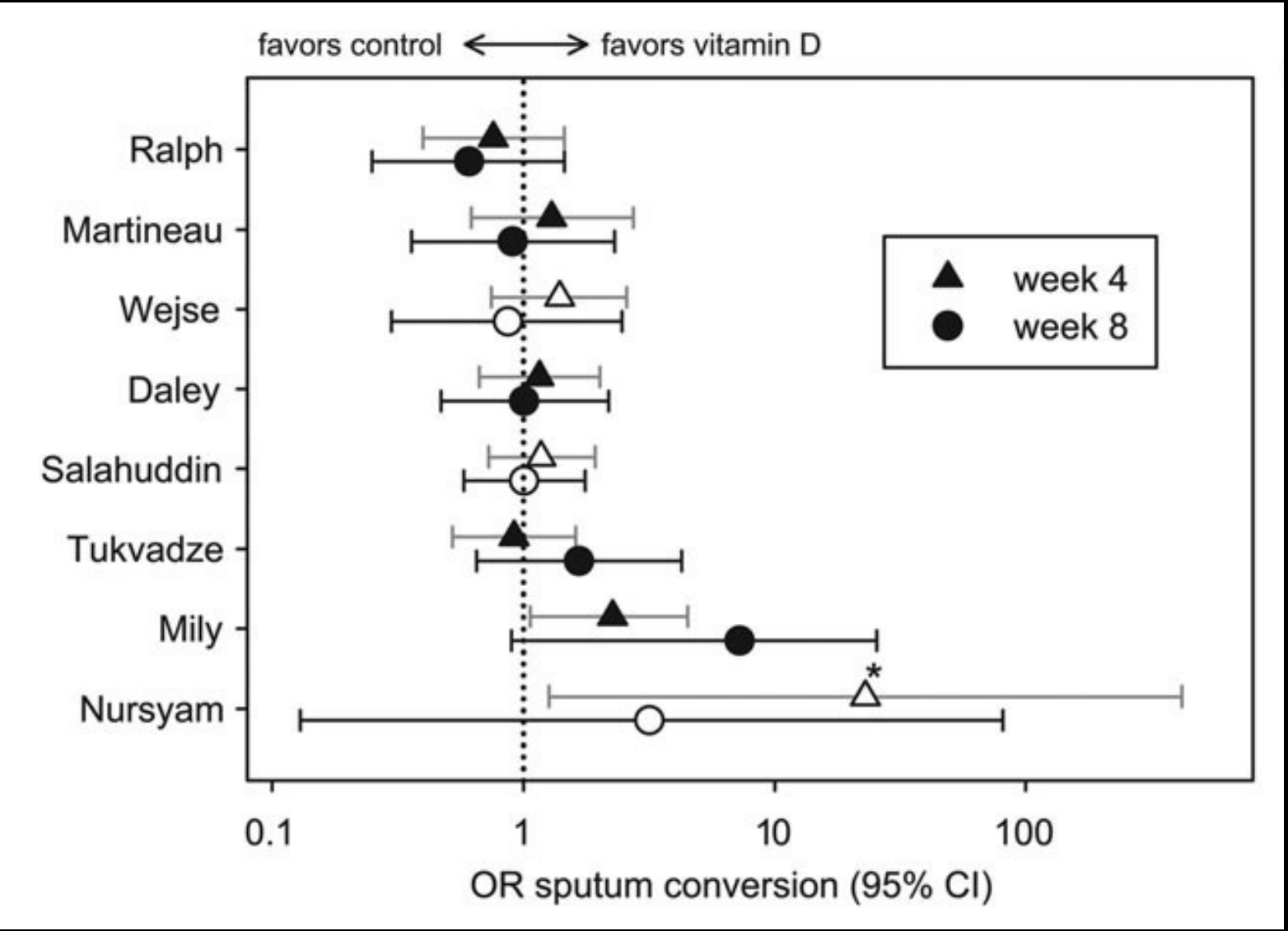
  sputum conversion

  outcome (mortality 15%)

High dose- VitD3 reduces the time to sputum culture conversion in patients with *TaqI* genotype of the VDR

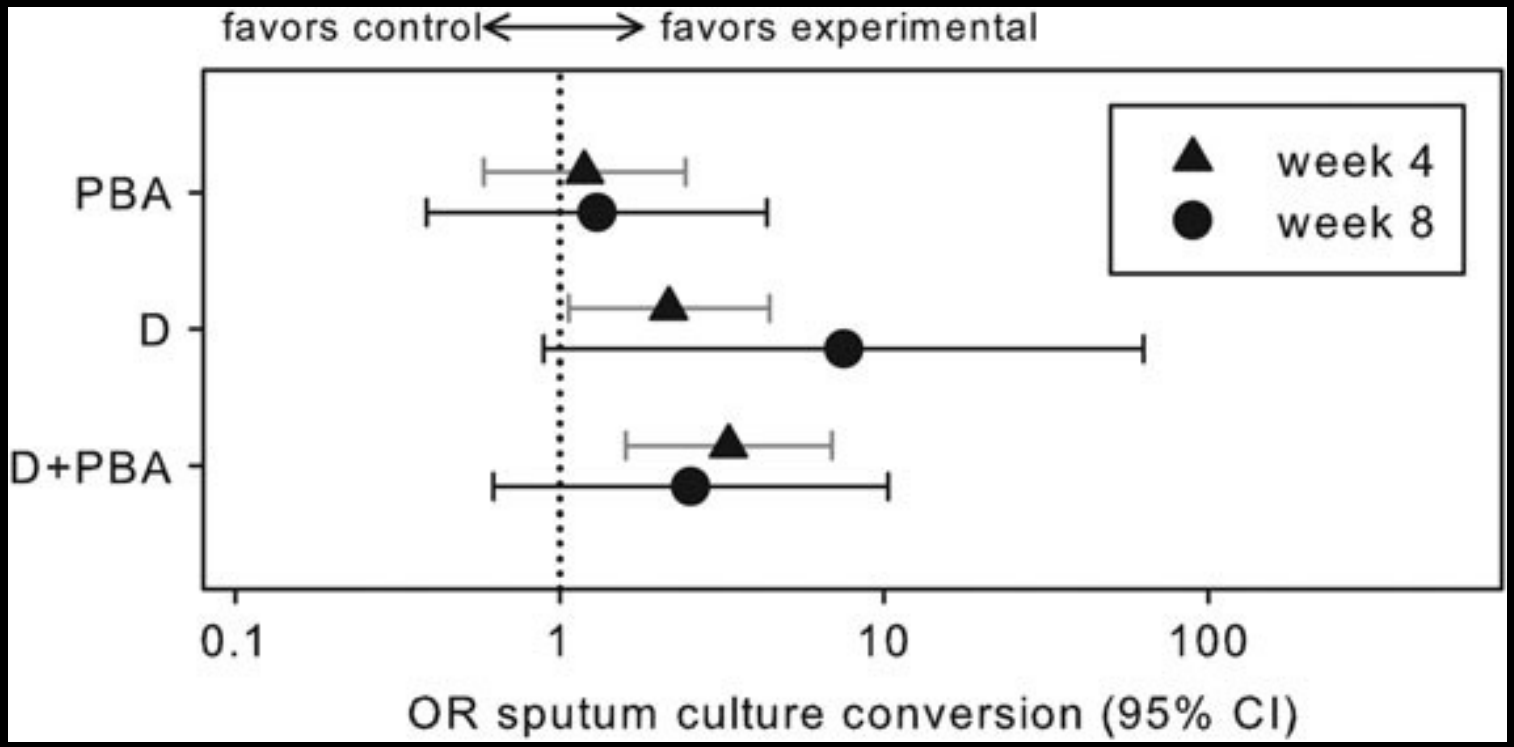
VDR genotype	p-value
TaqI	**0.03**
tt	**0.02**
Tt	**0.63**
TT	**0.71**
FokI	**0.85**

Vitamin D as Adjunctive Host Directed Therapy



Wallis and Zumla, Open Forum Infectious Diseases, DOI10:1093/ofid/ofw151

Vitamin D and Phenylbutyrate as Host Directed Therapy



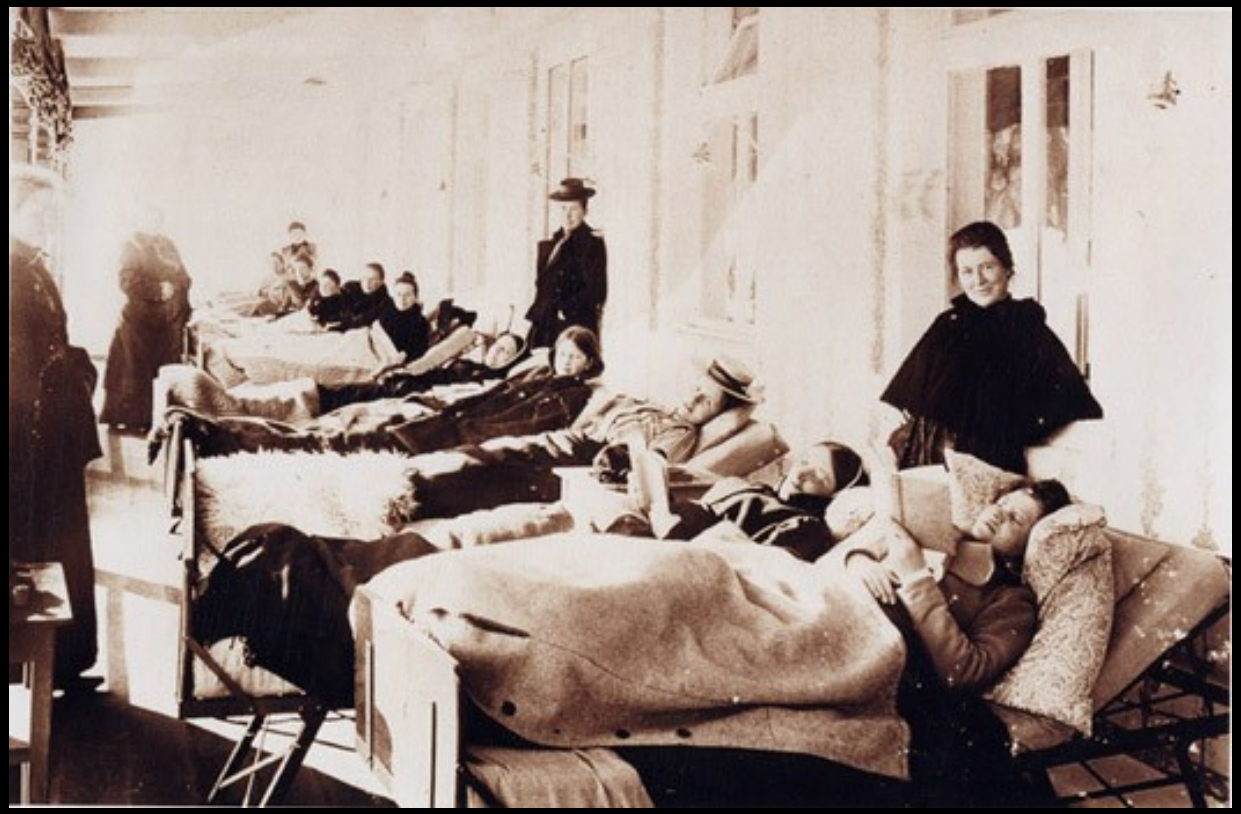
Wallis and Zumla, Open Forum Infectious Diseases, DOI10:1093/ofid/ofw151

Hermann Brehmer (1826-1889)

Biology student, that was cured from tuberculosis in the Himalaya region.

received his PhD for a thesis on „Tuberkulose ist eine heilbare Erkrankung“

Founded the world´s first sanatorium in 1854 in Silesia



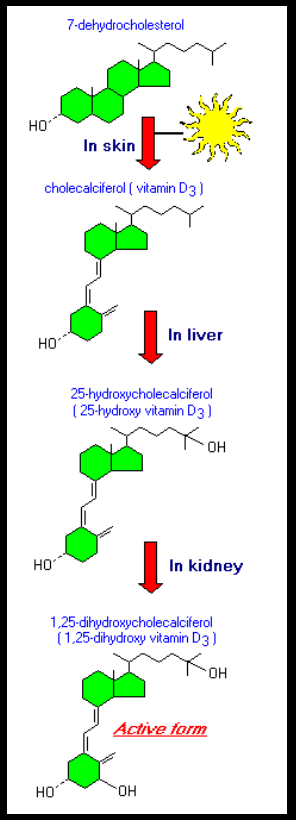


Fig 1 Hospital for Consumption and Diseases of the Chest, Brompton

Table 1| Results as shown in 1848 study

	Standard treatment	Standard treatment plus cod liver oil
Number of patients	542	535
Improved	60.8%	63.1%
Arrested	5.6%	18.1%
Deteriorated or died	33.3%	18.8%

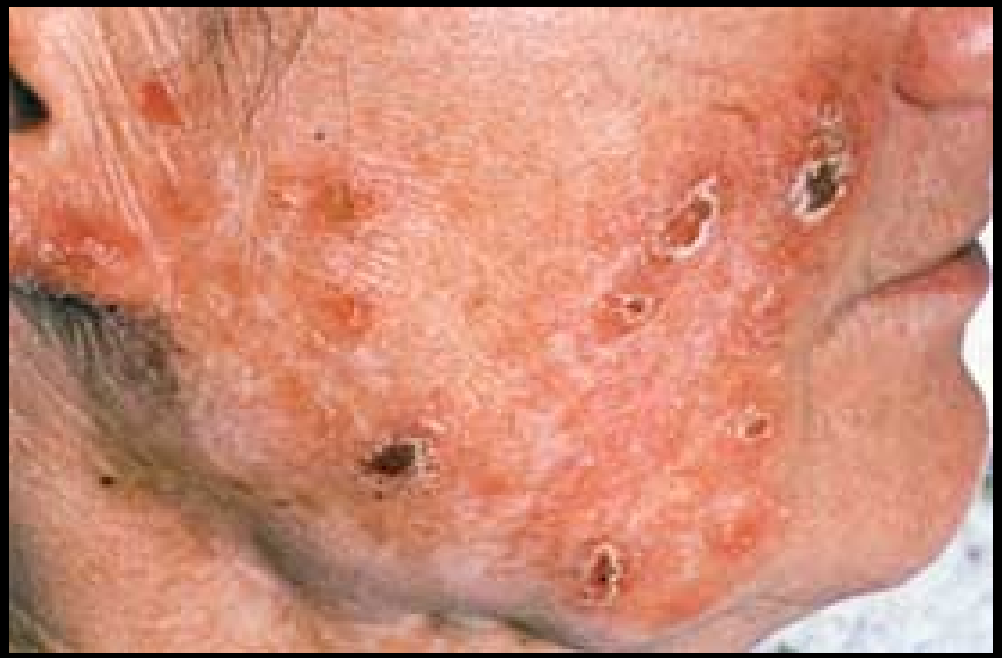
Green M., Brit Med J, 343: d7505, 2011

The first Nobel Prize for tuberculosis research goes to……..

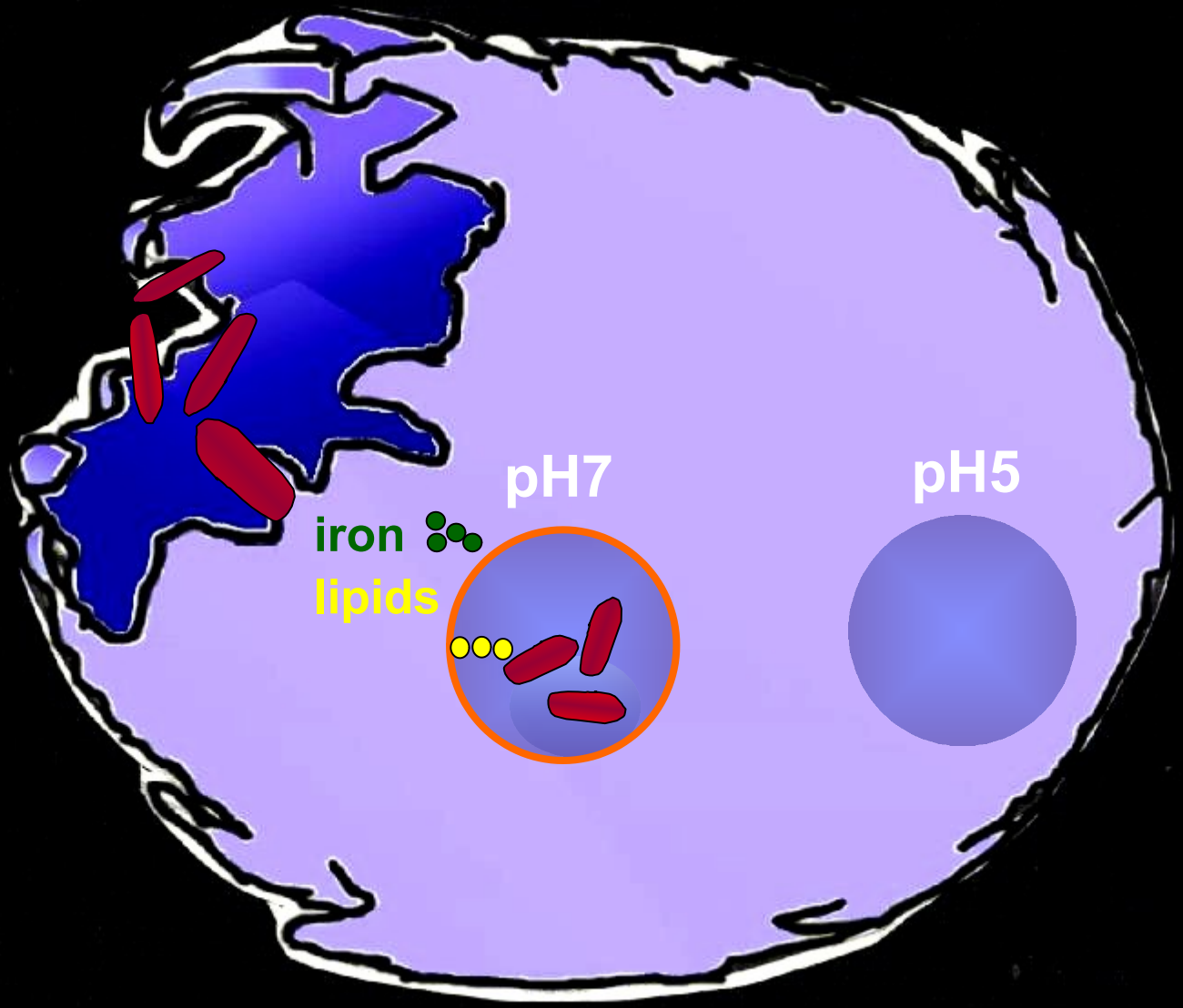
The first Nobel Prize for tuberculosis research goes to……..

Niels Ryberg Finsen (1860-1904)

1903: Nobel Prize for the introduction of UV therapy in the treatment for tuberculosis of the skin (Lupus vulgaris)



M. tuberculosis prevents acidification of lysosomes



Sciencexpress Report

Protein Kinase G from Pathogenic Mycobacteria Promotes Survival Within Macrophages

Anne Walburger,[1]* Anil Koul,[2]* Giorgio Ferrari,[1]* Liem Nguyen,[1]* Cristina Prescianotto-Baschong,[1] Kris Huygen,[3] Bert Klebl,[2] Charles Thompson,[1] Gerald Bacher,[2] Jean Pieters[1]†

[1]Biozentrum, University of Basel, Klingelbergstr. 50/70, CH-4056 Basel, Switzerland. [2]Axxima Pharmaceuticals AG, Max-Lebsche-Platz 32, 81377 Munich, Germany. [3]Pasteur Institute, Engelandstraat 642, B1180 Brussels, Belgium.

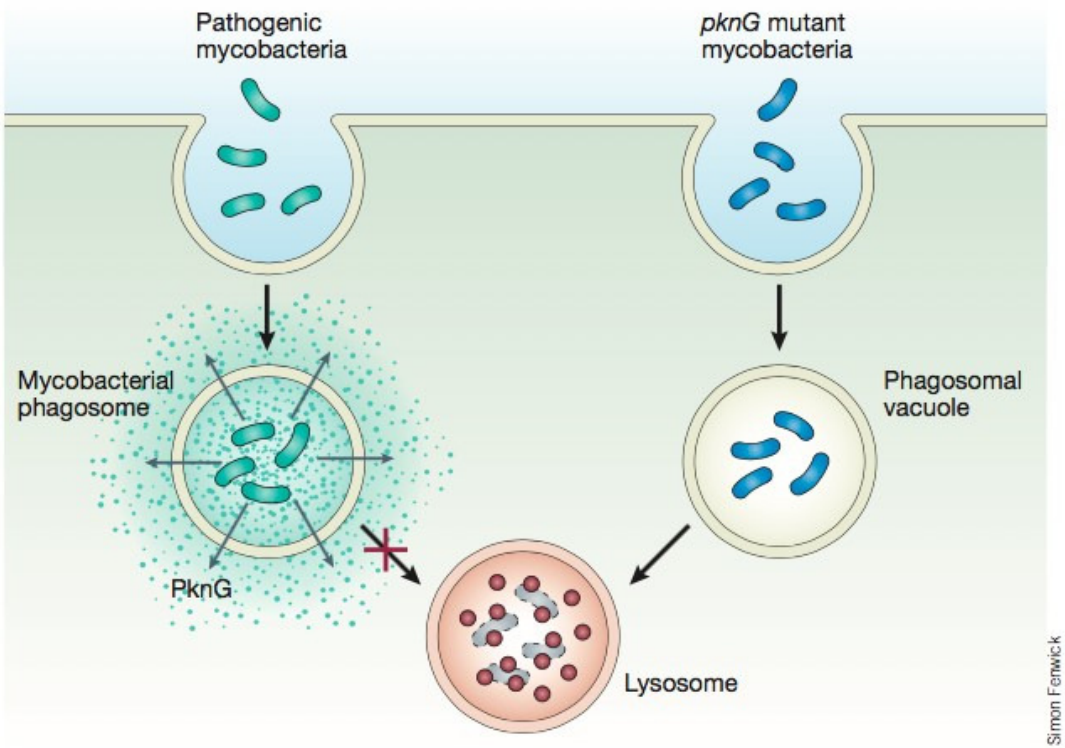
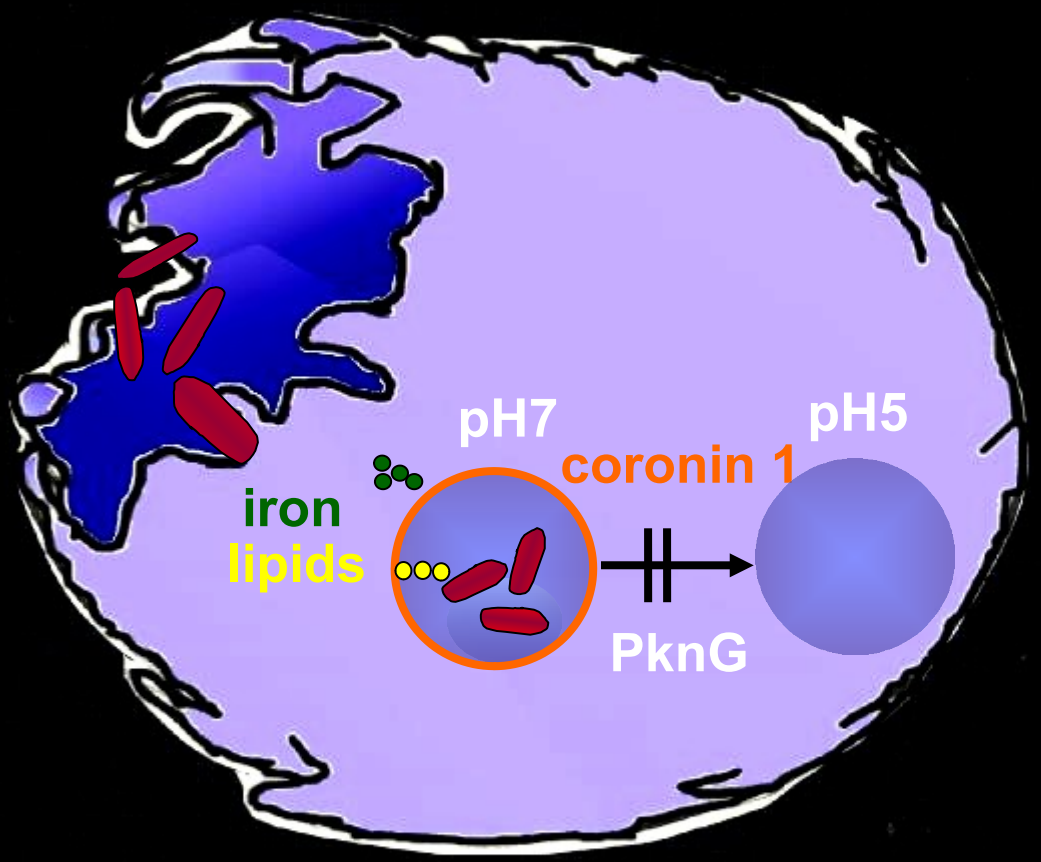


Figure 1 PknG affects the intracellular traffic of *M. tuberculosis* in macrophages. Most microbes and nonpathogenic mycobacteria quickly find themselves in lysosomes, where they are killed. By contrast, *M. tuberculosis* stays within phagosomes; the bacterium releases PknG to block phagosome-lysosome fusion. Bacteria lacking *pknG* are rapidly transferred to lysosomes and eliminated. Modified from ref. 20.

Walburger et al., Science, 304: 1800, 2004

Warner and Mizrahi, Nat Med, 13: 282, 2007

M. tuberculosis prevents acidification of lysosomes

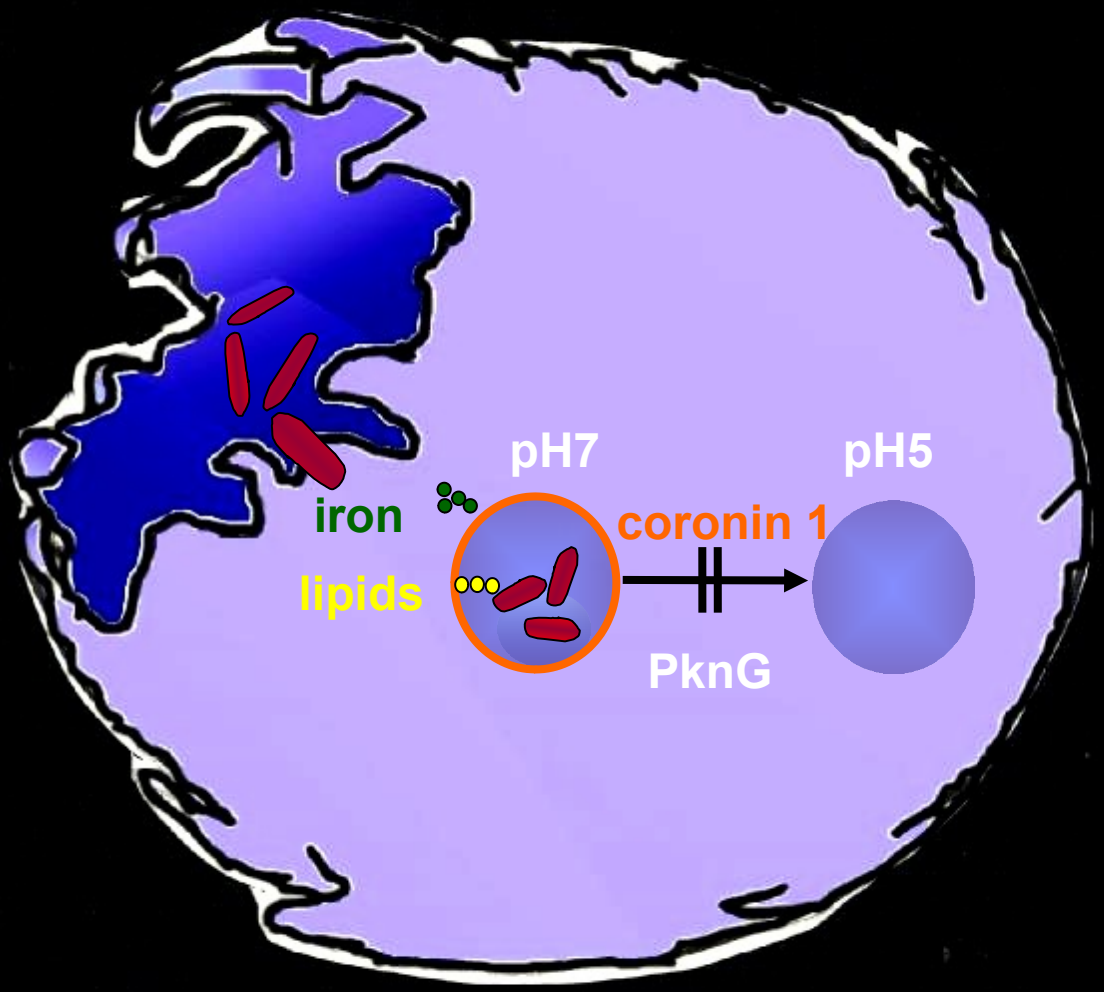


Therapeutic intervention: overcome acidification block

Example: interferon-γ (MacMicking, Russell, Deretic)

Problem: intolerable toxicity upon systemic administration

Search for small molecules capable of overcoming the *M. tuberculosis*-mediated maturation block



Abl tyrosine kinase: Role in immunity

- T cell development, signalling, proliferation, CD8+ T cellsexpansion and cytokine production[1]

- maturation of monocytes to dendritic cells[2]

- modulation of autophagy[2]

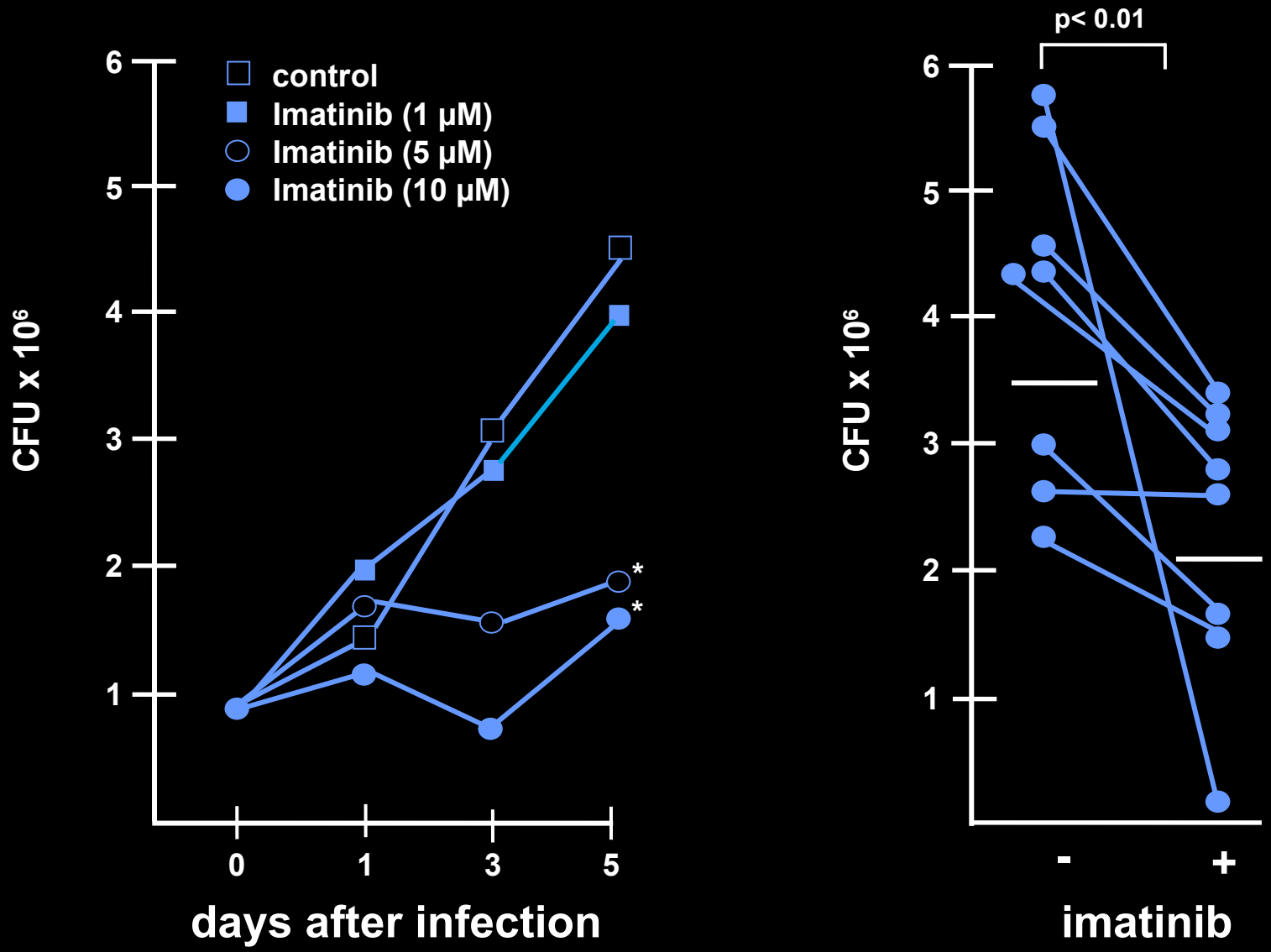
- modulation of trafficking and function of lysosomes[3]

1 Gu et al., Immunol. Rev., 228: 170, 2009

2 Appel et al., Blood, 103: 538, 2004

3 Yogalingam et al., J. Biol. Chem., 51: 35941, 2008

Pharmacological inhibition of Abl tyrosine kinase limits the growth of intracellular *M. tuberculosis*



Bruns et al., J Immunol, 189: 4069, 2012

Imatinib-Sensitive Tyrosine Kinases Regulate Mycobacterial Pathogenesis and Represent Therapeutic Targets against Tuberculosis

Ruth J. Napier,[1] Wasiulla Rafi,[5,6,7] Mani Cheruvu,[3,7] Kimberly R. Powell,[2] M. Analise Zaunbrecher,[1,3] William Bornmann,[4] Padmini Salgame,[5] Thomas M. Shinnick,[3] and Daniel Kalman[2,*]

Napier et al., Cell Host & Microbe, 10: 475, 2011

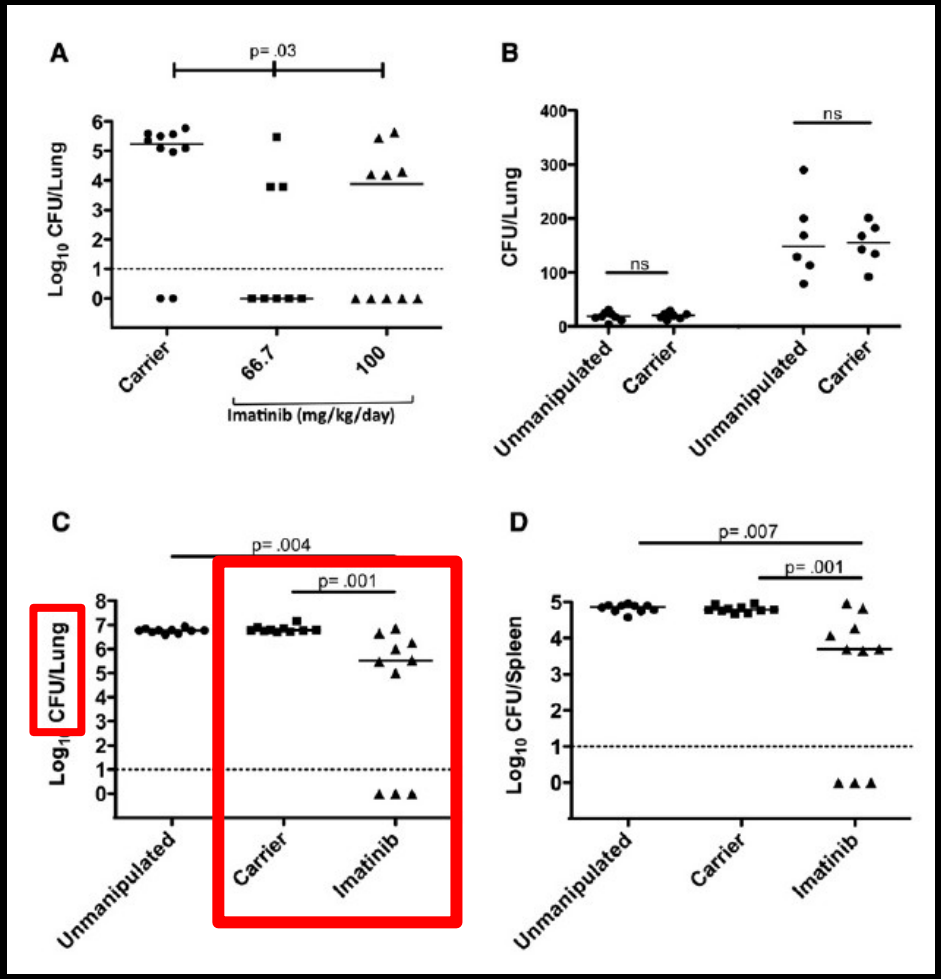


Figure 7. Imatinib Reduces Bacterial Load in Mice Infected with *Mycobacterium tuberculosis*

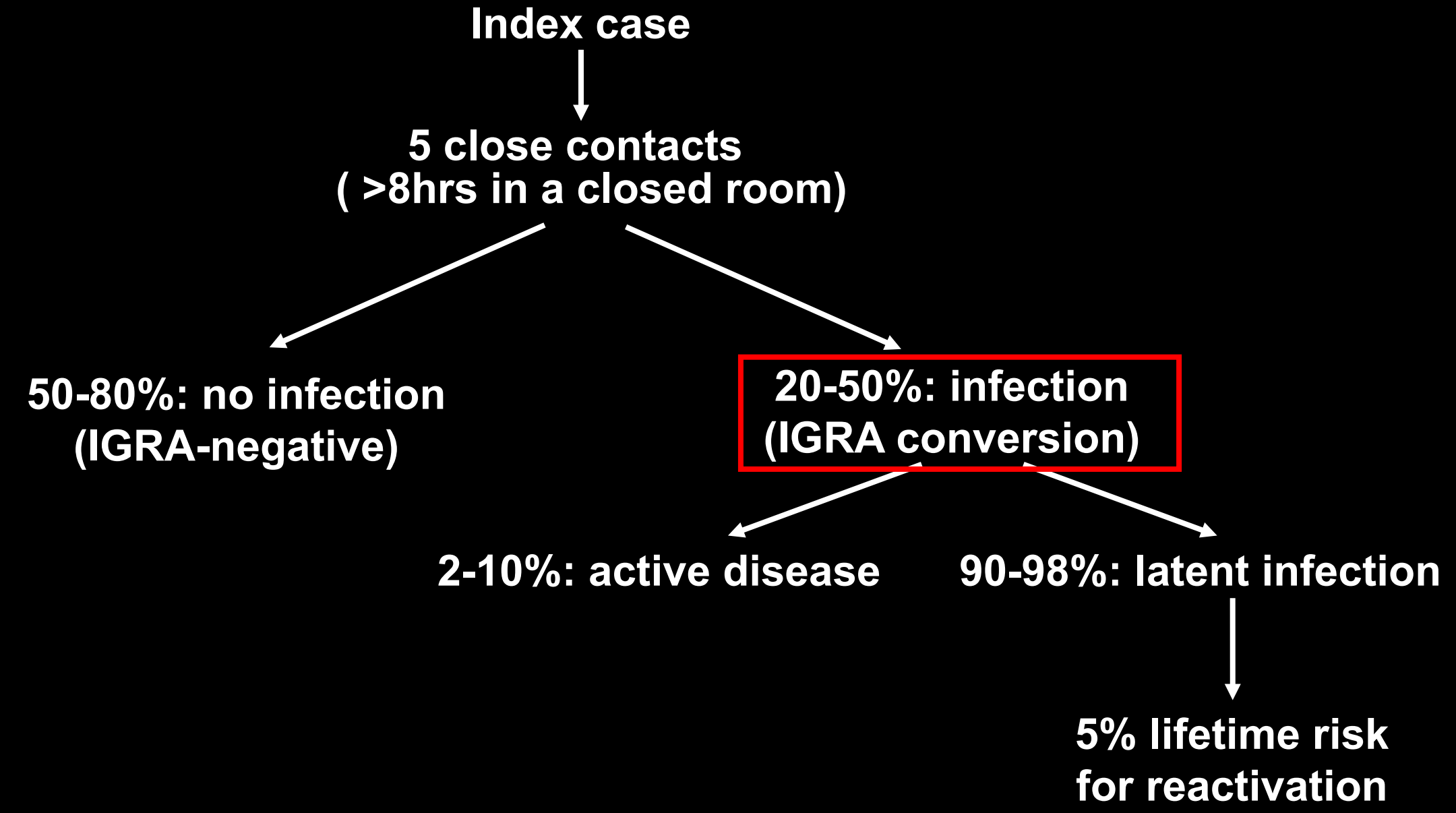
(A) C57Bl/6 mice were infected with 50–100 cfu of aerosolized *Mtb* Erdman. Beginning 24 h prior to infection, animals were administered water (carrier) or imatinib at concentrations of 66.7 mg/kg/day or 100 mg/kg/day. Cfu was determined in right superior lobe of the lung at 28 days p.i. Solid lines represent the median cfu; dotted line represents the limit of detection (10 cfu); p values were determined by a nonparametric Kruskal-Wallis test.

(B) C57Bl/6 mice were administered carrier pumps 24 h prior to infection. Unmanipulated or carrier-treated mice were infected with a low (2.5×10^5 cfu; left) or high dose (1×10^7 cfu; right) of aerosolized *Mtb* Erdman and cfu determined in the whole lung at 24 h p.i. The solid line represents the median cfu.

(C and D) C57Bl/6 mice were infected with 2.5×10^5 cfu of aerosolized Mtb Erdman. Beginning 24 h prior to infection, animals were either left untreated or administered carrier (water) or imatinib at a concentration of 66.7 mg/kg/day. Cfu was determined by plating homogenates of the whole lung (C) or spleen (D) at 28 days p.i. The solid line represents the median cfu; dotted line represents the limit of detection (10 cfu); p values were determined by a nonparametric Mann-Whitney test.

Delivery of Drugs In Vivo

Innate immunity alone fails to prevent infection with *Mycobacterium tuberculosis* in 20-50% of the cases

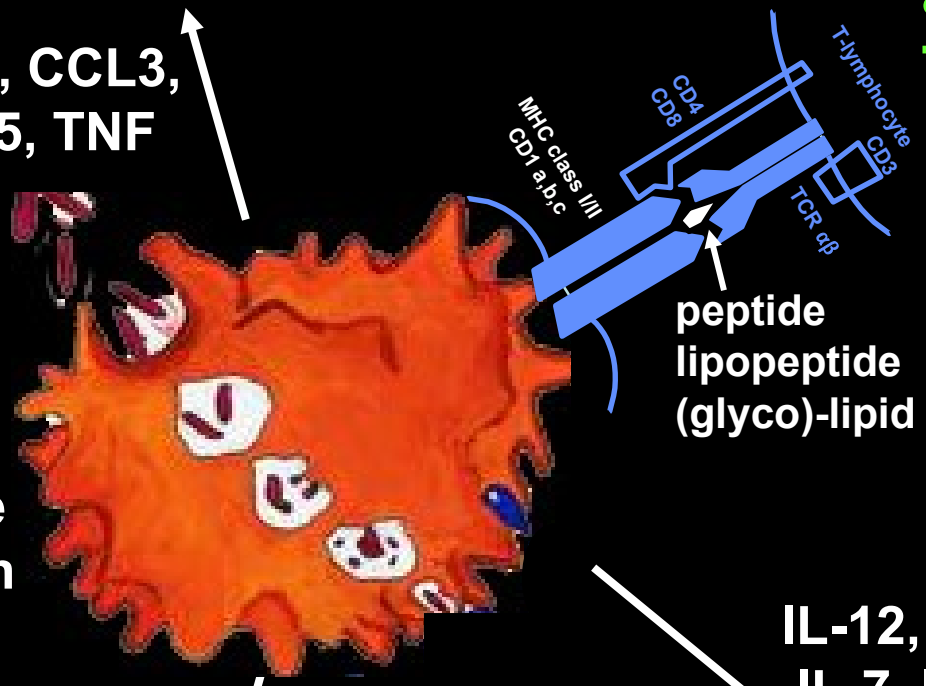


Adaptive immunity to *Mycobacterium tuberculosis*

T-cell-recruitment

CCL-2, CCL3,
CCL-5, TNF

antigen-specific T-cell-activation



antimicrobial activity

nitric oxide
cathelicidin
autophagy

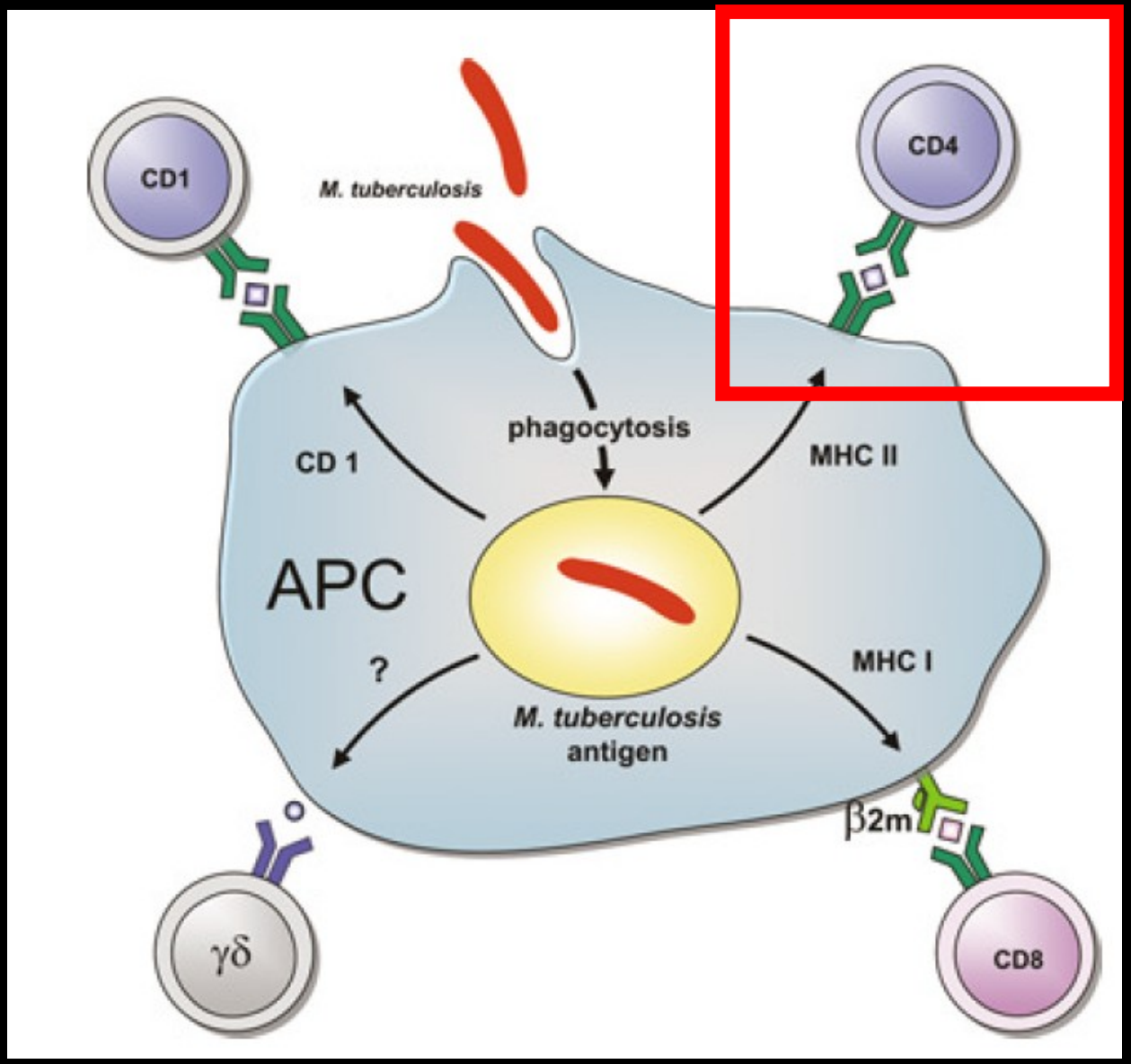
IL-12, IL-18, IL-23
IL-7, IL-15, IL-21

IL-10, TGF-β

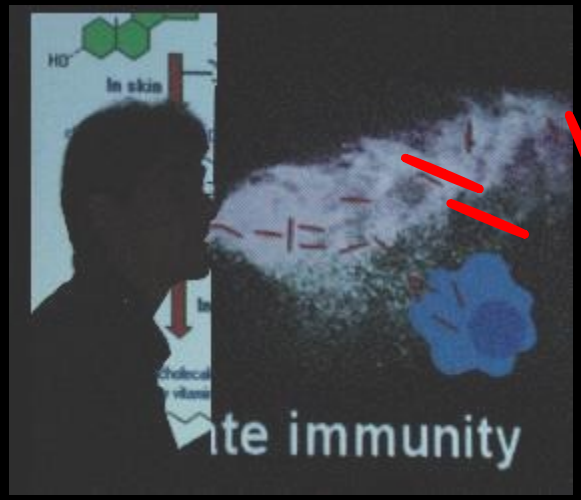
regulatory T-cells

Th1-response
Th17-response
cytotoxic T cells

Major T cell subsets in human tuberculosis



Kaufmann et al., Int J Tuberc Lung Dis, 10: 1068, 2006



TNF

Fc receptors
complement receptors
scavenger receptors
TLR 2/4
DC-SIGN

innate immunity

MHC molecules

CD1

peptide

lipid or lipoglycan

TCR

TCR

CD3

CD3

acquired immunity

CD4+ T helper cell

CD8+ cytolytic T cell

IL-2

IFN-γ
TNF

granuloma

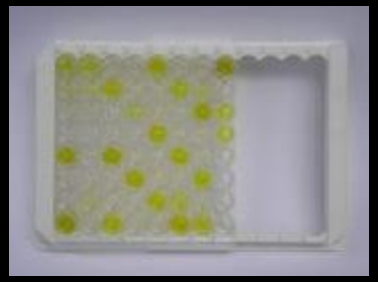
effector (memory) cells

Natural course of infection with Myobacterium tuberculosis

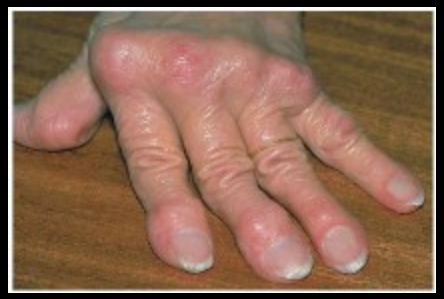
infection

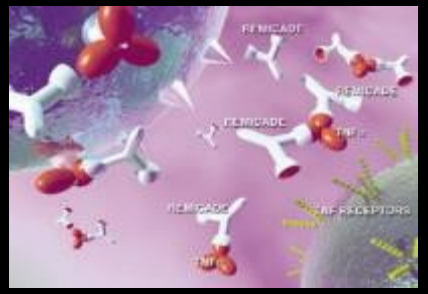
5%: active TB

95%: latent TB



Treatment of autoinflammatory diseases with TNF antibodies



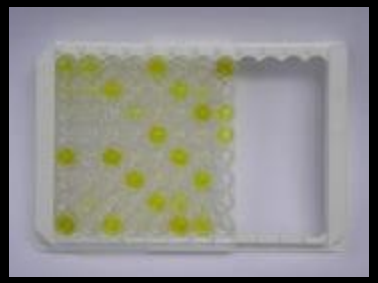


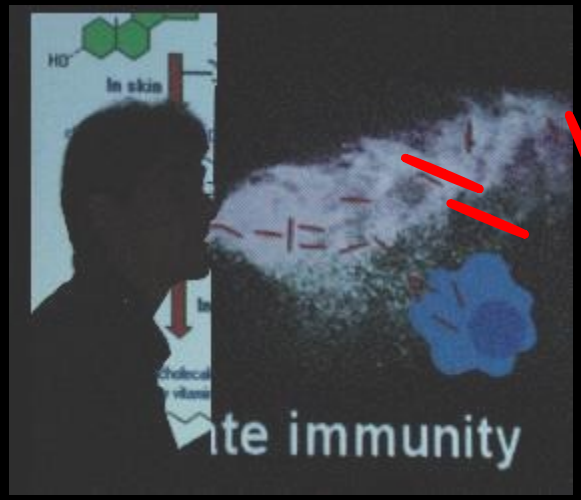
infection

rheumatoid arthritis

anti TNF therapy

5%: active TB
95%: latent TB





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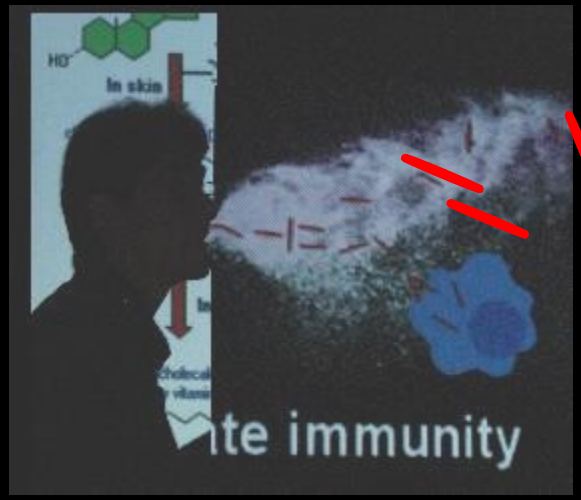

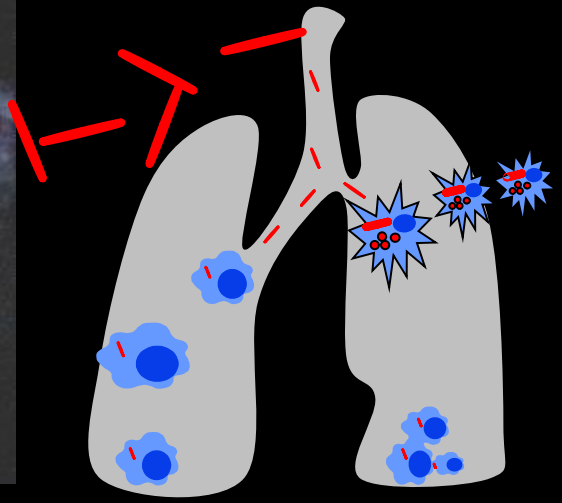

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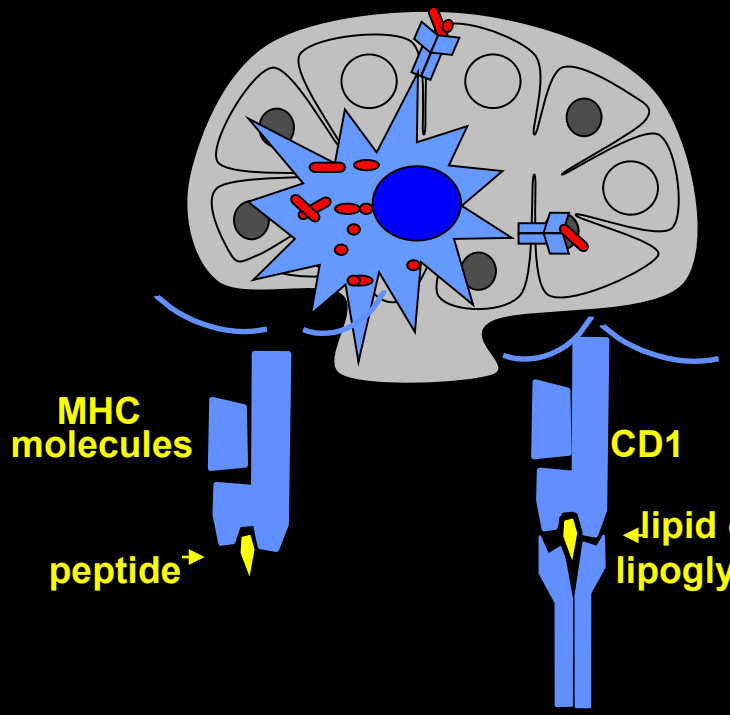

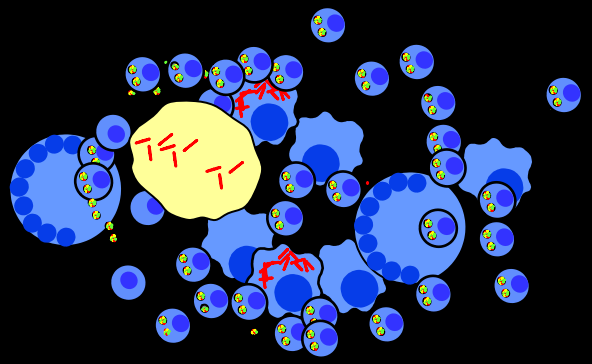

acquired immunity

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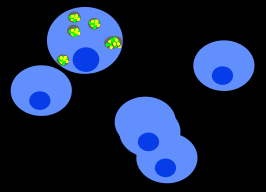
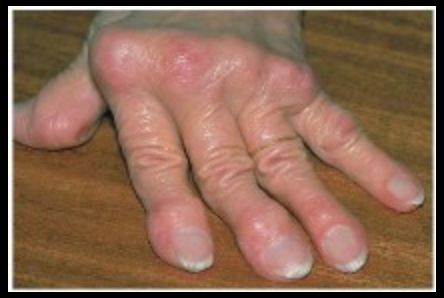
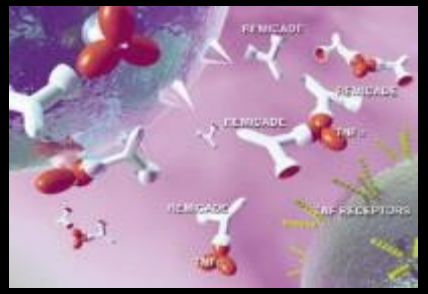


effector cells

Reactivation of tuberculosis during Infliximab therapy





infection

rheumatoid arthritis

anti TNF therapy

reactivation

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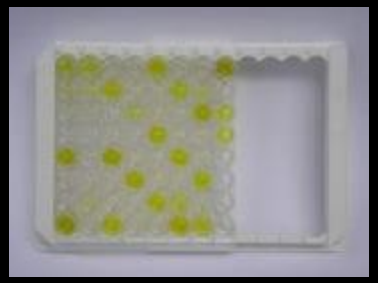
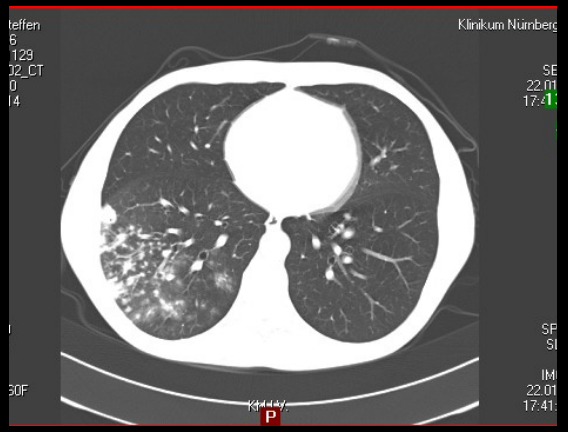
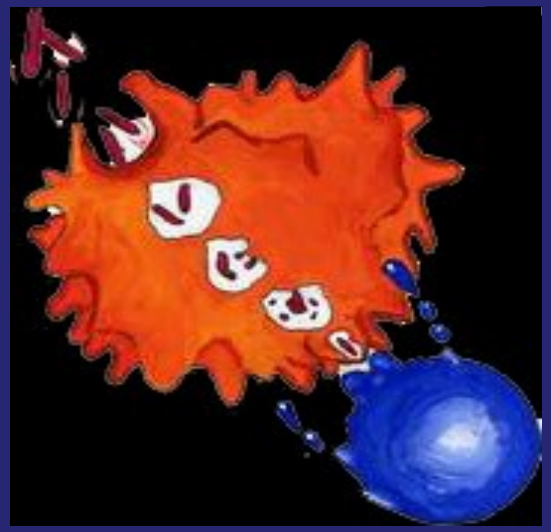




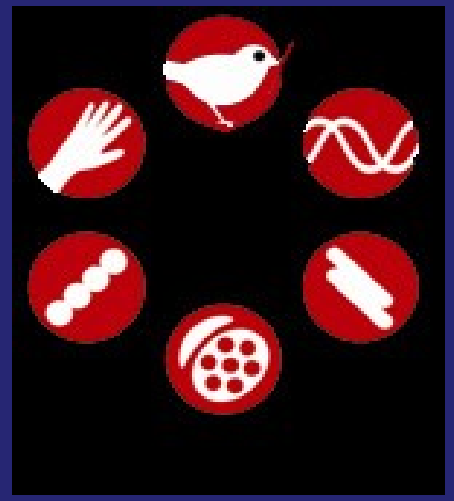


Steffen Stenger

Medical Microbiology and Hygiene

University Hospital Ulm

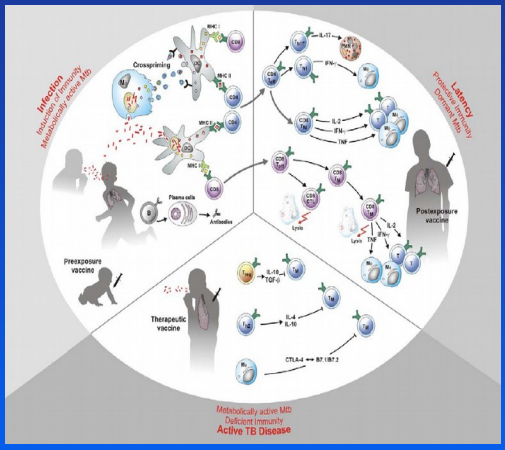
MyTB Lab



From Basic Immunology to an efficient Vaccine (?)

Albert Calmette
(1863-1933)

Camille Guerin
(1872-1961)



Available Vaccines for Humans

- rubella
- measles
- mumps
- chicken pox
- influenza
- Hepatitis A
- Hepatitis B
- rabies
- polio
- yellow fever
- tick-borne encephalitis
- japanese encephalitis
- rotavirus
- papilloma virus

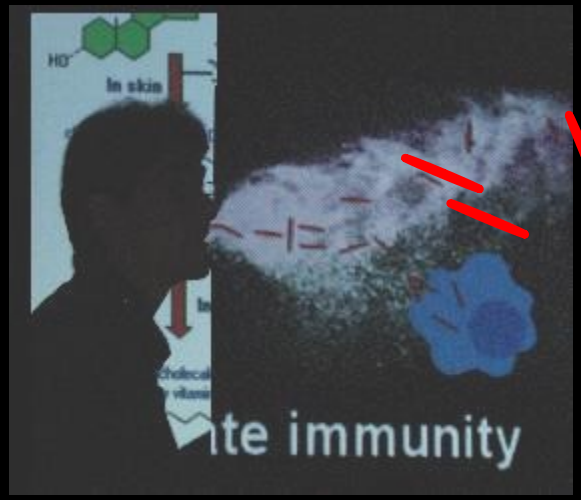
viral diseases:

protection by neutralizing antibodies

Available Vaccines against Bacterial Infections

toxoid	polysaccharide/protein conjugate
- tetanus	- meningococci
- diphtheria	- pneumococci
- pertussis	- Haemophilus influenza B
	- typhoid fever

protection based on neutralizing antibodies directed against toxins or the bacterial capsule



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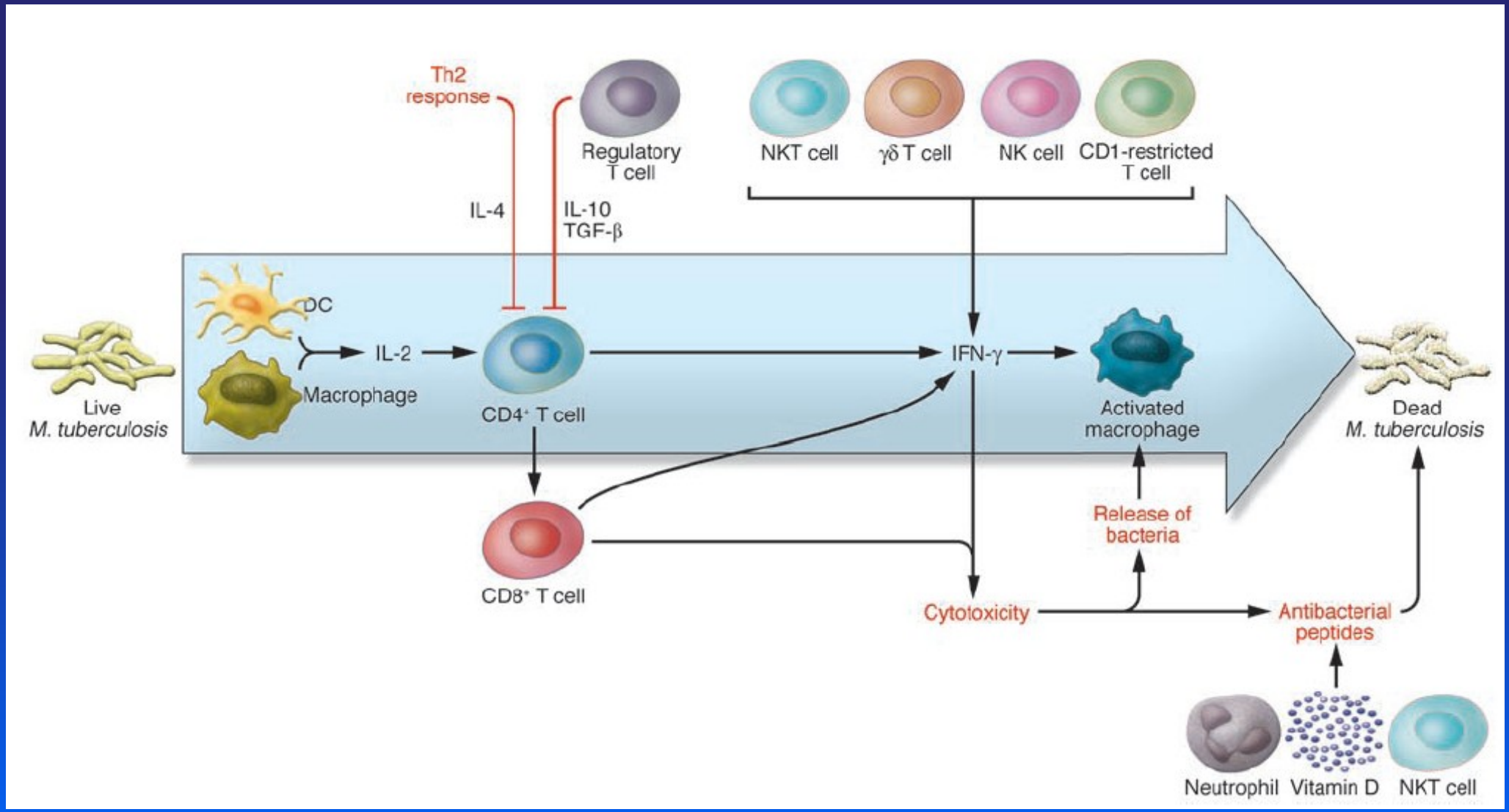
Tuberculosis: The Ideal Vaccine



Young et al., J Clin Invest., 118: 1255, 2008

Prevention of Tuberculosis: BCG Vaccination

- 1900: Institute Pasteur in Lille, Albert Calmette / Camille Guerin

- subculture in ox bile reduced virulence in guinea pigs
- 1902: strain from a tuberculous cow provided by Nocard
- 1908: subculture of the strain from 1902 in 3-week intervals
- 1913: initiation of a vaccine trial in cattle

Luca and Mihaescu, Maedica, 8: 53, 2013

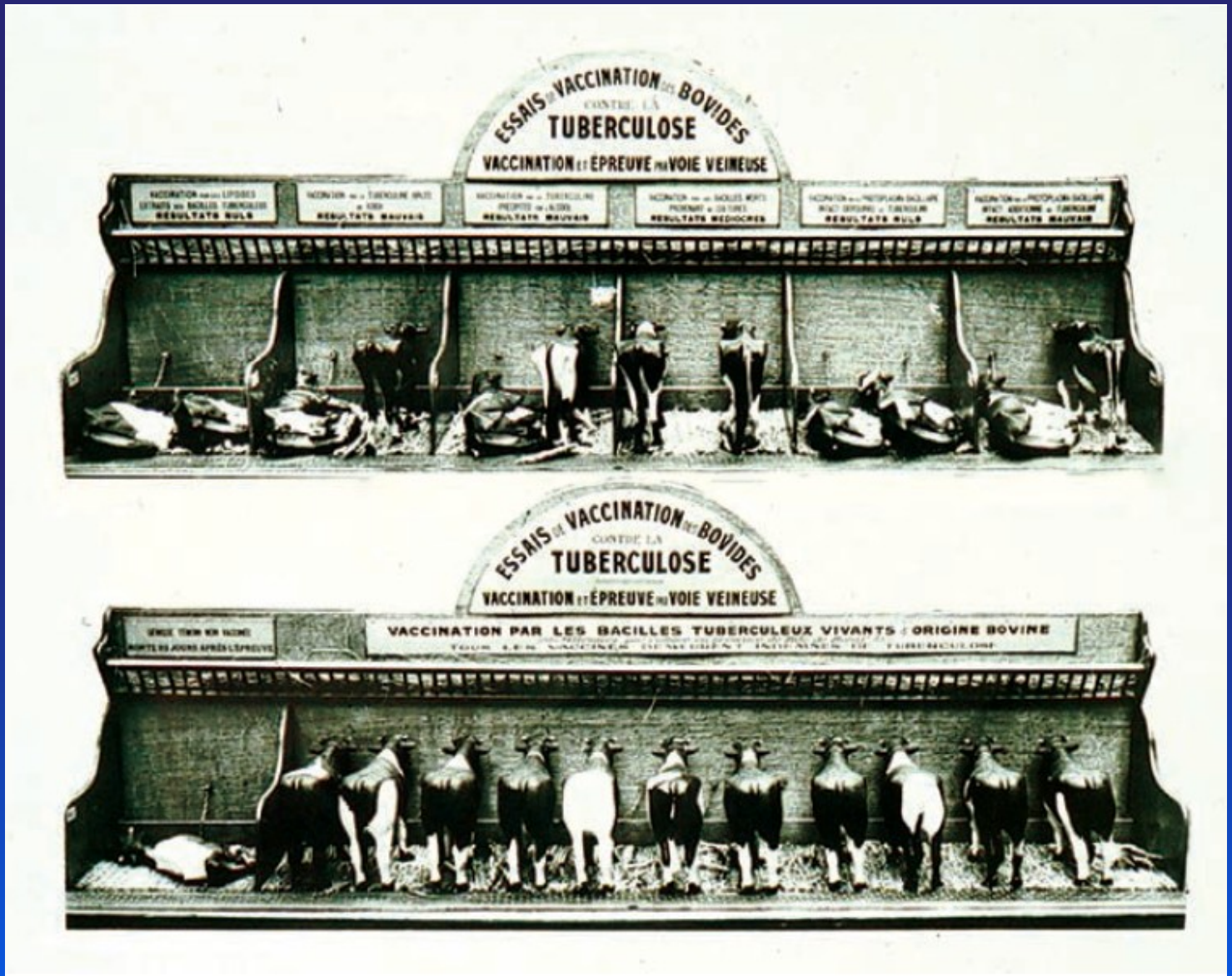


Figure 5.1.1 Comparison of protective efficacy of live BCG with that of other vaccine preparations in cattle

The Lübeck Accident

251 infants were BCG (provided by Guerin)
vaccinated in 1929 and 1930

72 (29%) died of tuberculosis within 2-5 months

135 (54%) developed tuberculosis, but recovered

44 (17%) became tuberculin-positive, but remained well

1931/1932:
Prof. Georg Deycke,
Dr. Ernst Altstaedt and
Anna Schütze were
convicted for man slaughter

Prevention of Tuberculosis: BCG Vaccination

- 1930: increasing criticism against Calmette and Guerin even though *BCG* was not directly responsible for the „Lübecker Impfunglück“

Figure 5.1.4 Caricature of Albert Calmette around the vaccination controversy

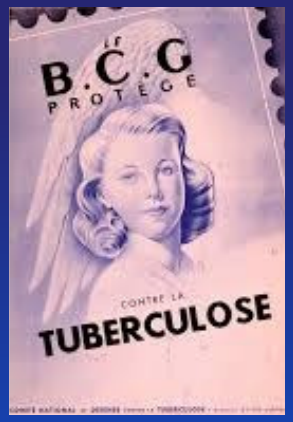
Note: Albert Calmette not only worked on BCG but had also been very active in the development of anti-snake venom antiserum, as well as in developing methods to purify sewage sludge.

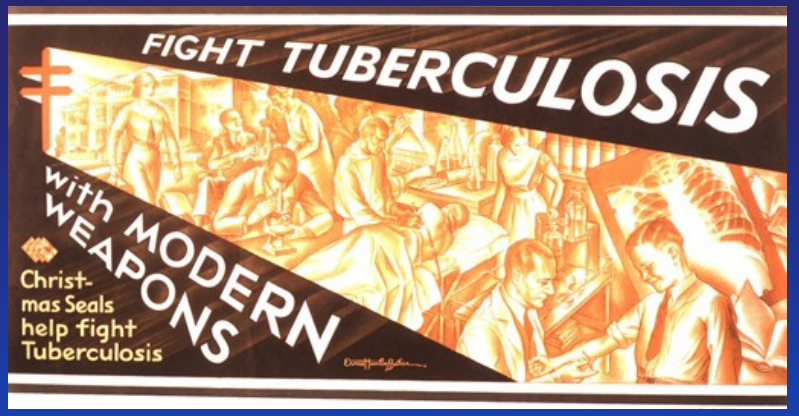
Prevention of Tuberculosis: BCG Vaccination

- 1930: increasing criticism against Calmette and Guerin even though *BCG* was not directly responsible for the „Lübecker Impfunglück“

- 1940s: spread and propagation of *BCG* by UNICEF, WHO

Figure 5.1.3 Recommendations for vaccination with BCG in France





- 1950s: major clinical trials in United Kingdom (Copenhagen strain, succesful) and United States (Tice strain, unsuccesful)

- most countries in the world implemented *BCG* vaccination

- most widely used vaccine world wide (4 billion doses)

BCG-Vaccination: Efficacy

Haiti 1973	80% protection
Great Britain 1977	75% protection
Chingleput 1980	no protection
Georgia, Alabama 1969	no protection
Northern Malawi 1992	no protection

Protection from severe disease in childhood,
but not from infectious pulmonary tuberculosis

Reasons for Variable Efficacy of BCG

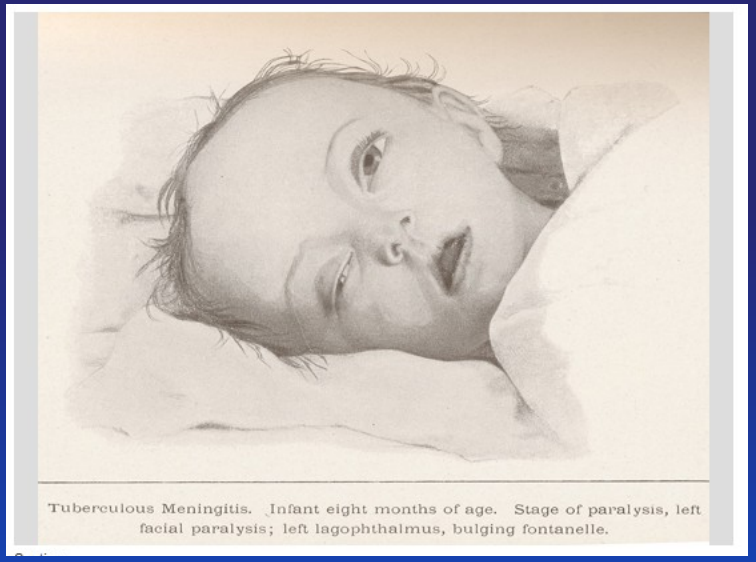
- variable exposure to environmental mycobacteria
- co-infections (helminths)
- nutritional status (iron, vitamin D)
- ethnicity, genetic background, immune status (HIV!)
- BCG is not clonal: different strains at different sites
- different quality of BCG (viability per dose)

BCG: Advantages

1. protection against severe disease in children

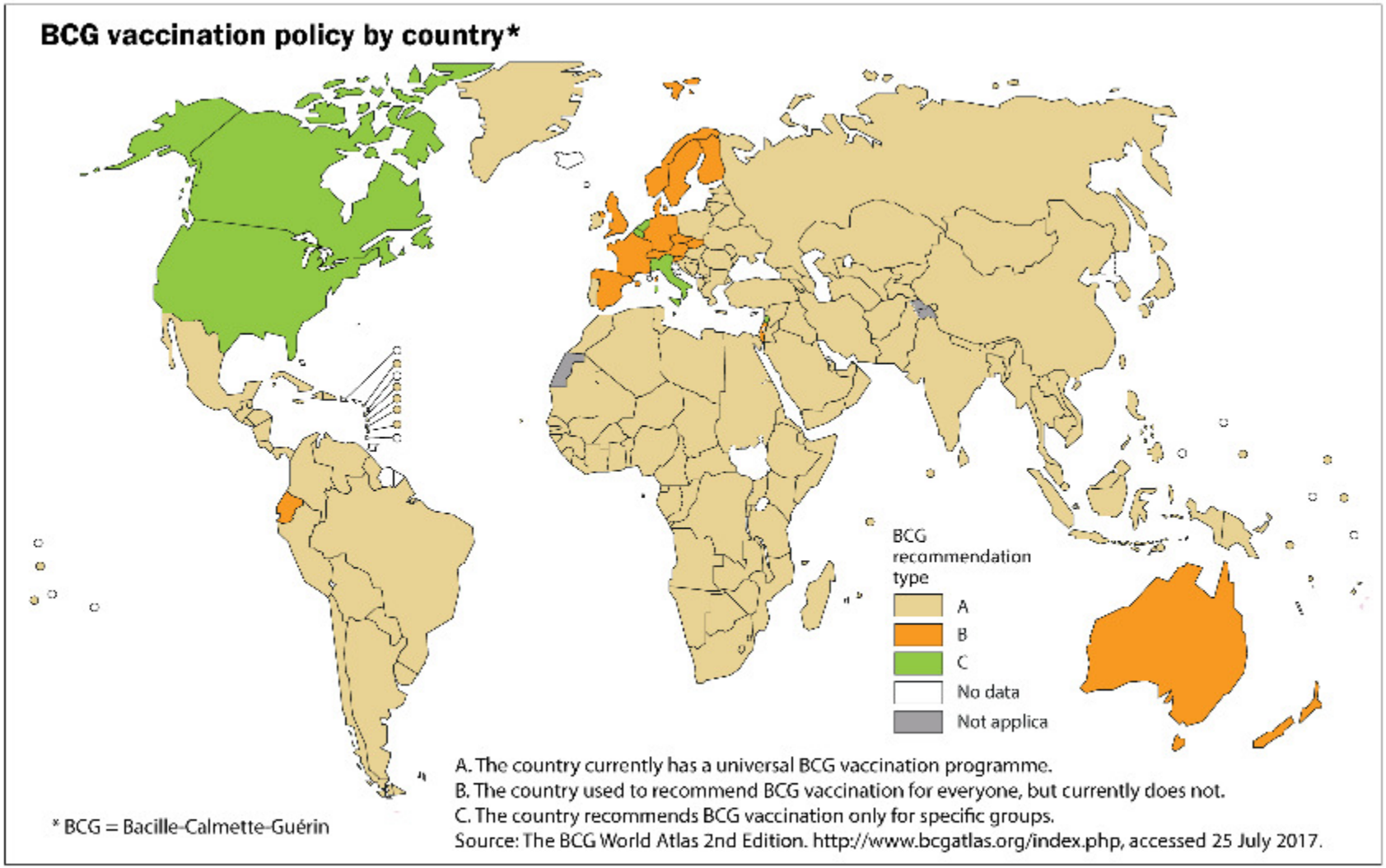
2. generally well-tolerated

3. practical: single dose to newborns



Tuberculous Meningitis. Infant eight months of age. Stage of paralysis, left facial paralysis; left lagophthalmus, bulging fontanelle.

BCG vaccination policy by country



Data Source: *Global Tuberculosis Report 2017*. WHO, 2017.

World Health Organization

Vaccine approaches: Examples

1. Purified lipid antigens
2. Genetically modified *M. smegmatis* („IKEPLUS“)
3. Genetically modified *BCG* (rBCGUreC:Hly, Aeras-422)

Urease-deficient BCG expressing listeriolysin

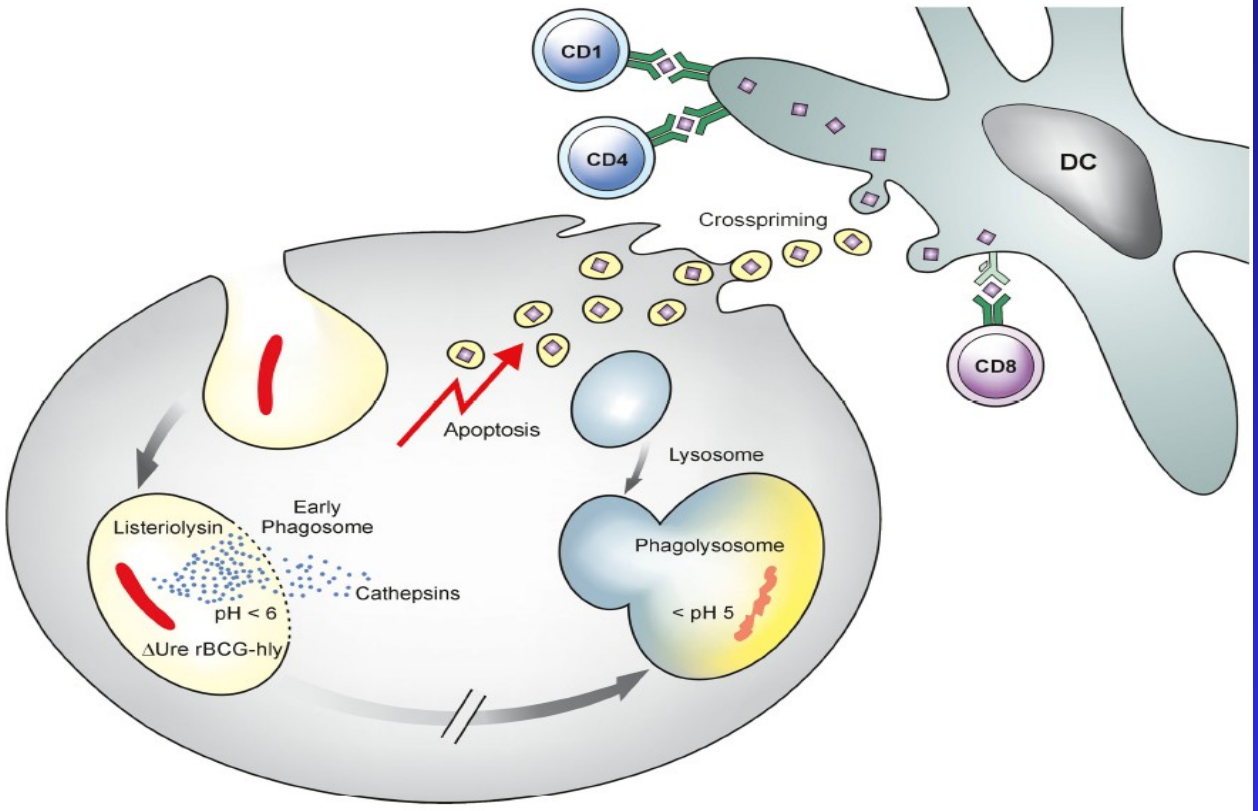


Figure 2. Schematic description of the underlying mechanism of improved T cell stimulation by a novel BCG vaccine. Recombinant BCG deleted in urease and expressing listeriolysin is capable of inducing a more profound immune response than wild-type BCG. The likely mechanism involves perforation of the phagosomal membrane, which allows leakage into the cytosol of both mycobacterial antigens and phagosomal enzymes such as cathepsins. Cathepsins are known to induce apoptosis. Thus, the new BCG vaccine strain induces crosspriming leading to a more efficacious immune response. Image provided by Stefan Kaufmann (Berlin, Germany) (25).

Kaufmann et al., J Exp Med, 201: 1693, 2005

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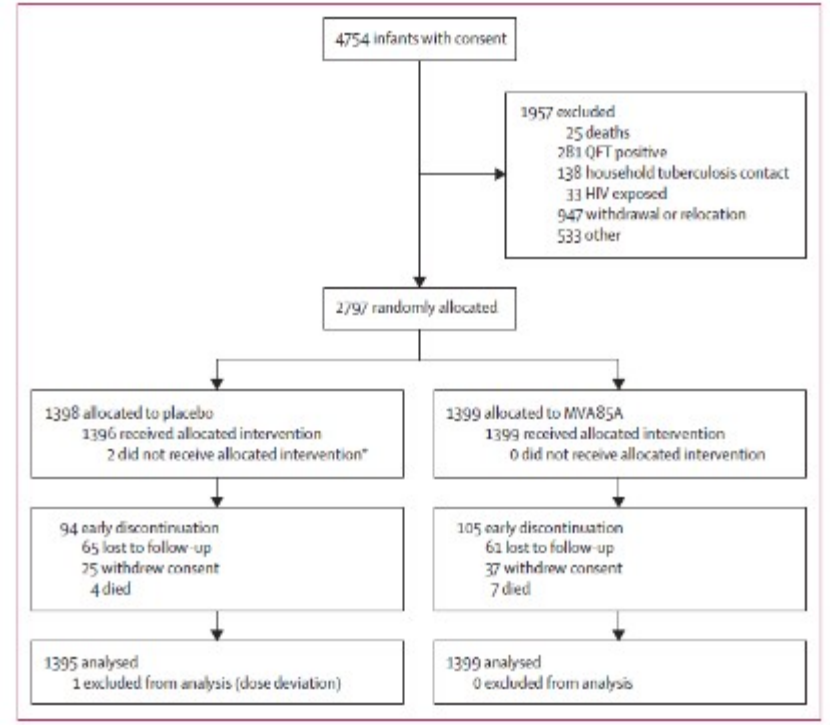
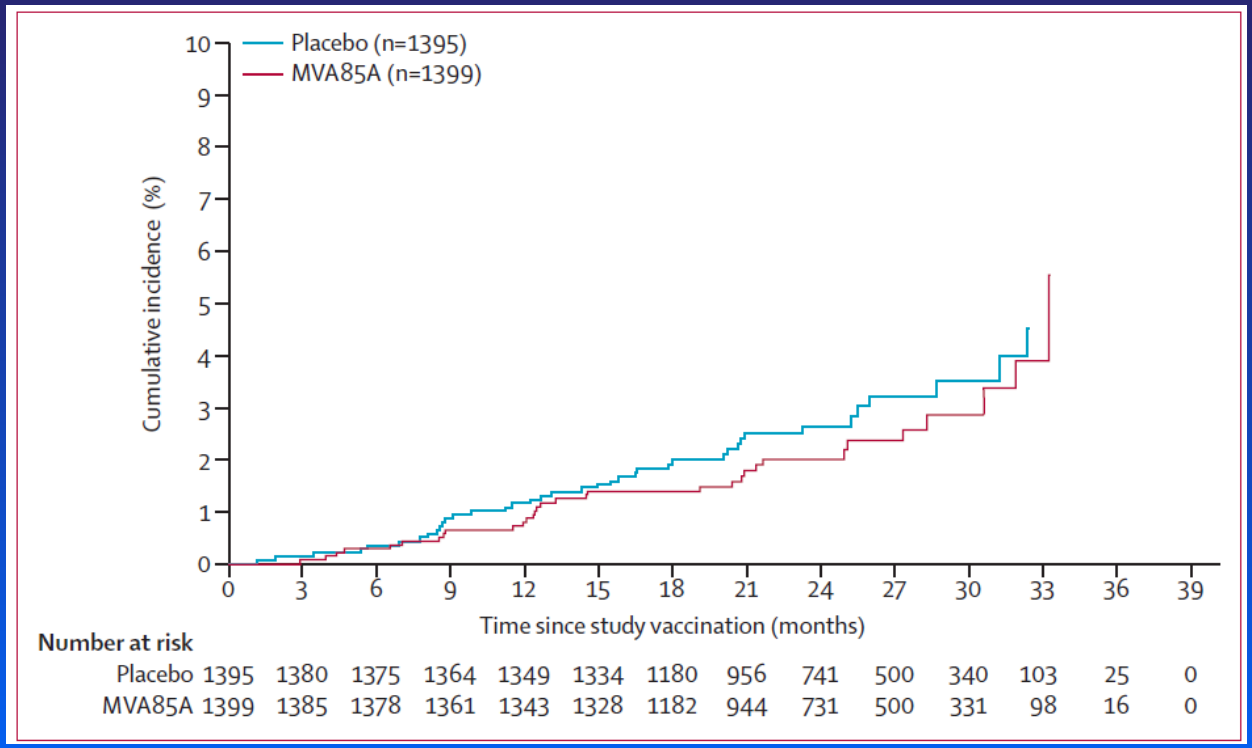
4. Genetically modified *M. tuberculosis* (PhoP mutant)
5. Proteins expressed in viral vectors (MVA85A)

Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial

Michele D Tameris, Mark Hatherill*, Bernard S Landry, Thomas J Scriba, Margaret Ann Snowden, Stephen Lockhart, Jacqueline E Shea, J Bruce McClain, Gregory D Hussey, Willem A Hanekom, Hassan Mahomed†, Helen McShane†, and the MVA85A 020 Trial Study Team*





Lancet, 381: 1021, 2013

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5. Proteins expressed in viral vectors (MVA85A)
6. Fusion proteins in adjuvant (H56 in IC31)

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Prevention of *M. tuberculosis* Infection with H4:IC31 Vaccine or BCG Revaccination

E. Nemes, H. Geldenhuys, V. Rozot, K.T. Rutkowski, F. Ratangee, N. Bilek, S. Mabwe, L. Makhethe, M. Erasmus, A. Toefy, H. Mulenga, W.A. Hanekom, S.G. Self, L.-G. Bekker, R. Ryall,* S. Gurunathan, C.A. DiazGranados, P. Andersen, I. Kromann, T. Evans, R.D. Ellis, B. Landry, D.A. Hokey, R. Hopkins, A.M. Ginsberg, T.J. Scriba, and M. Hatherill, for the C-040-404 Study Team†

N Engl J Med, 379: 138, 2018

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Phase 2b Controlled Trial of M72/AS01$_E$ Vaccine to Prevent Tuberculosis

O. Van Der Meeren, M. Hatherill, V. Nduba, R.J. Wilkinson, M. Muyoyeta, E. Van Brakel, H.M. Ayles, G. Henostroza, F. Thienemann, T.J. Scriba, A. Diacon, G.L. Blatner, M.-A. Demoitié, M. Tameris, M. Malahleha, J.C. Innes, E. Hellström, N. Martinson, T. Singh, E.J. Akite, A. Khatoon Azam, A. Bollaerts, A.M. Ginsberg, T.G. Evans, P. Gillard, and D.R. Tait

N Engl J Med DOI 10. 1056/NEJMoa1803484

Towards an improved tuberculosis vaccine: Perspective

- at present the problem is not the availabilty of vaccine candidates

- bottle neck: number of vaccines that can be tested in clinical efficacy trials given the limited clinical trial capacity world wide

- desperately needed: surrogate endpoint markers as correlates of protection to reduce the need for long term, large scale clinical trials

- development of predictive animal models

- establish improved routes of delivery e.g. aerosol