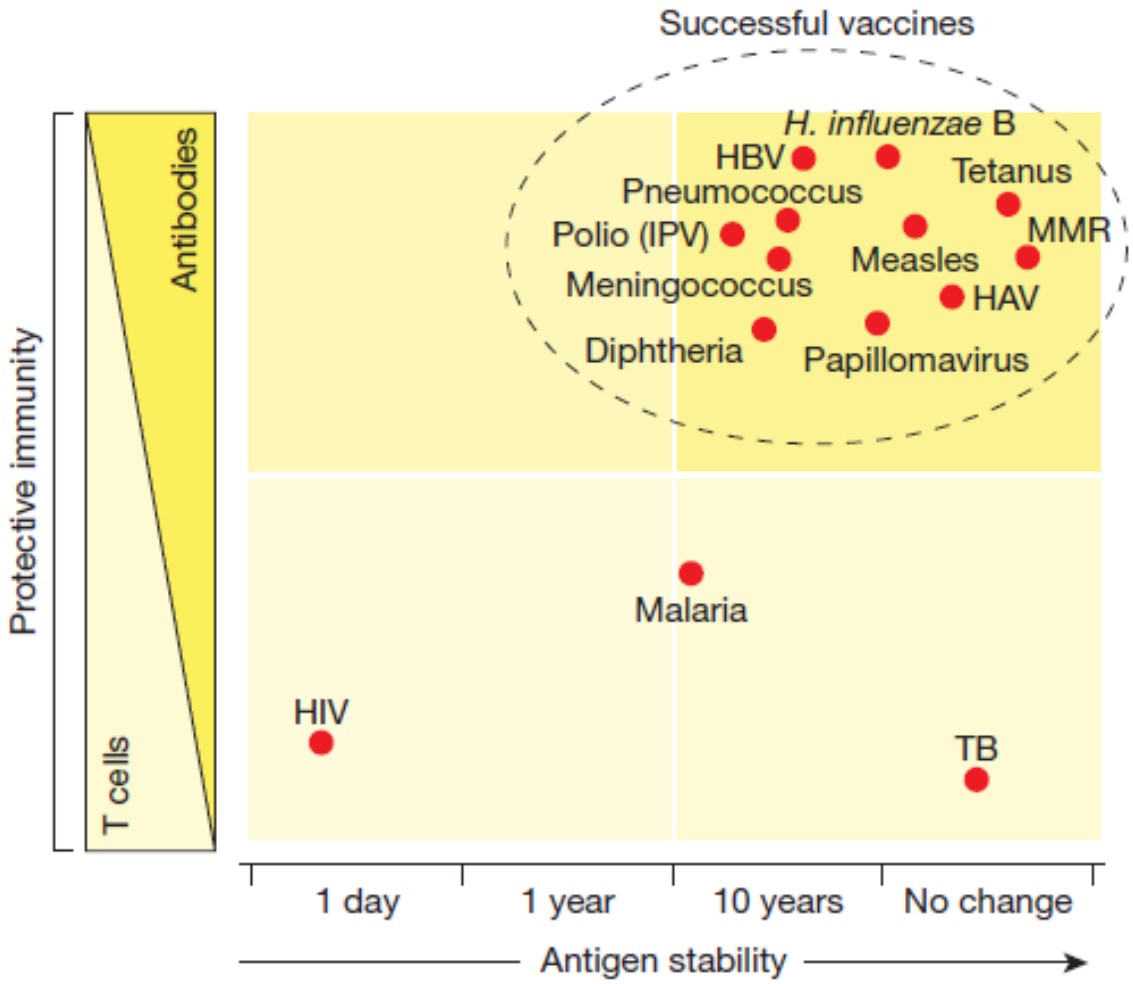
Perspective in novel TB vaccine development

Mohamed Ridha BARBOUCHE M.D., Ph.D.
Department of Immunology
Institut Pasteur de Tunis

Challenging infectious diseases



2011 | VOL 473 | NATURE

Existing TB Vaccine is not effective for global TB epidemic control

BCG introduced in 1921

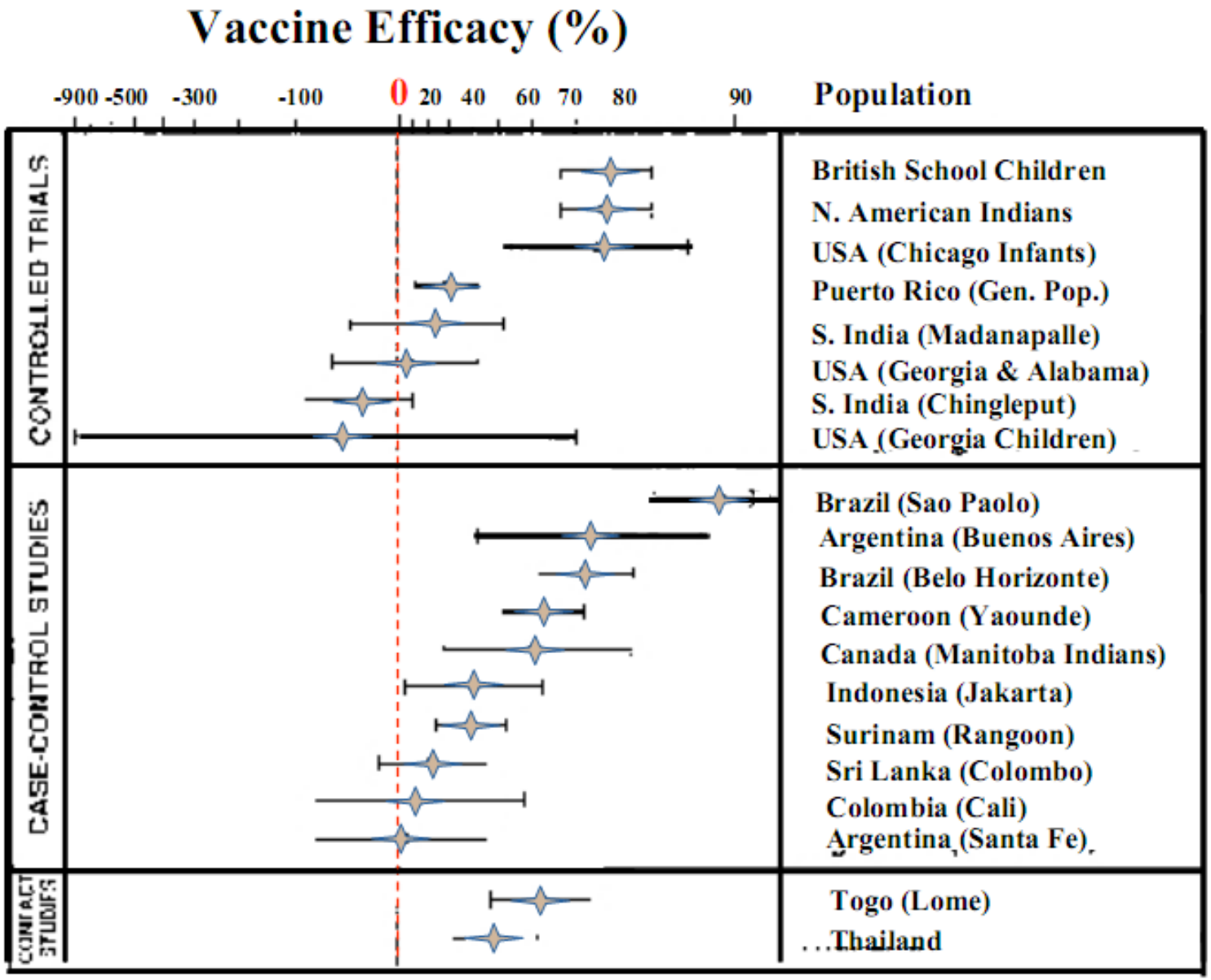
BCG unreliable against pulmonary TB, which accounts for most TB disease worldwide.

BCG is not known to protect against latent TB.

BCG is not recommended for use in infants infected with HIV due to increased risk for severe BCG -related complications.

Despite wide use, BCG has had no apparent impact on the growing global TB epidemic.

BCG does reduce risk of severe pediatric TB disease, so it should continue to be used until a better TB vaccine is available.



The ‘’Koch phenomenon”

Discovery of MTB in 1882

He attempted to develop a therapy using a sterile filtrate from in vitro culture of MTB.

This was tested in active TB patients and was spectacularly unsuccessful. The inflammatory responses induced were severe.

The necrotic reaction or ‘’Koch phenomenon” is now known to be due to overproduction of pro-inflammatory cytokines (particularly TNFα).

But, we also know that inflammatory response is required for successful protection against TB.

Immunology of TB is complex

- **Koch's failure represents a key point for TB vaccine development: ''the same immune responses are involved in both protection and disease''.**
- **Dissecting complex interplay between host immune system and mycobacteria is critical to build effective strategies of vaccination.**
- **Understanding how to fine tune immune responses in MTB naïve and infected individuals is a pre-requisite as well as identifying markers of resistance, latency, protection and disease is required.**
- **Due to high frequency of latent infection in the developing world, stimulating protection without worsening pathology is the goal of current attempts to develop novel TB vaccines.**

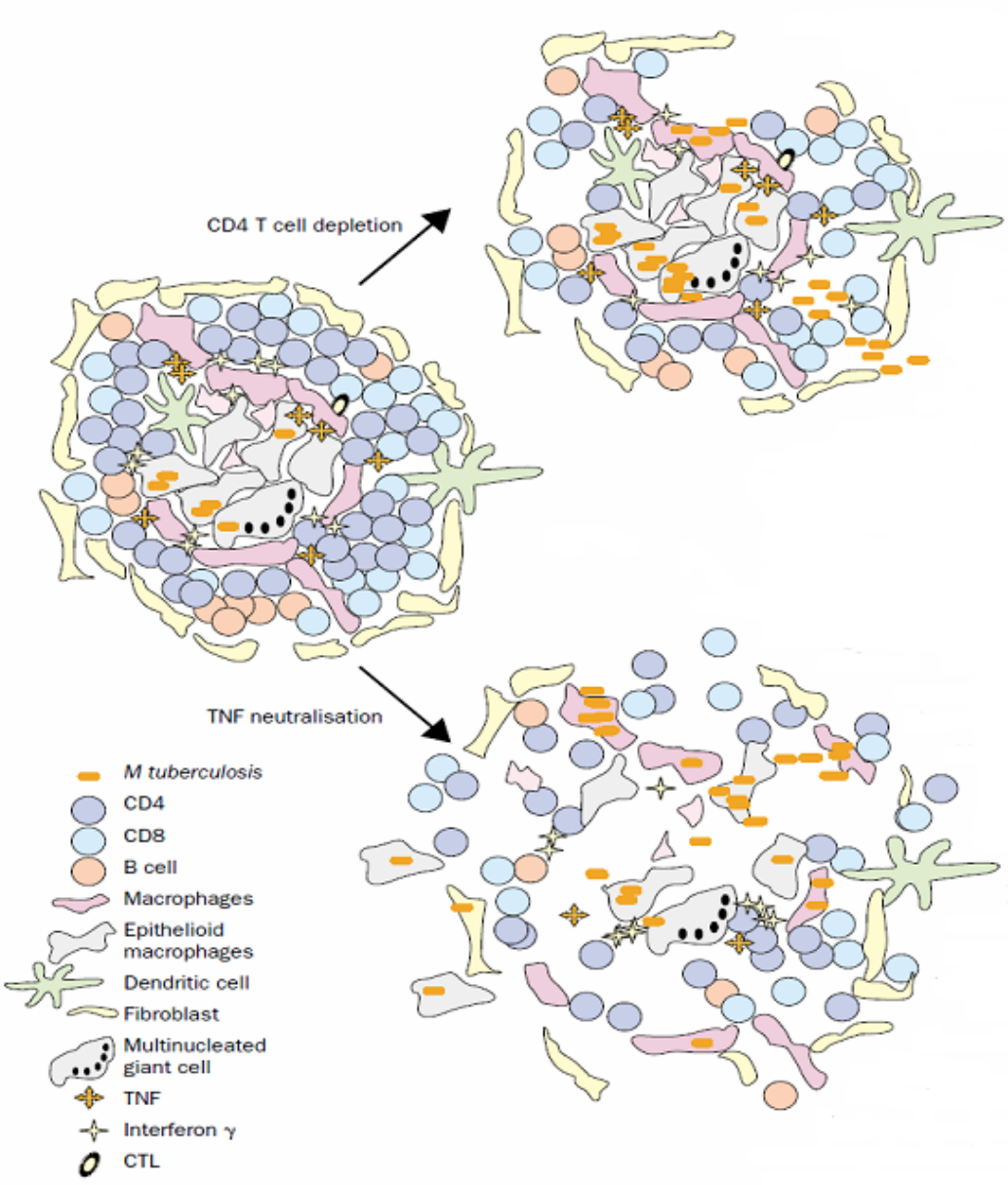
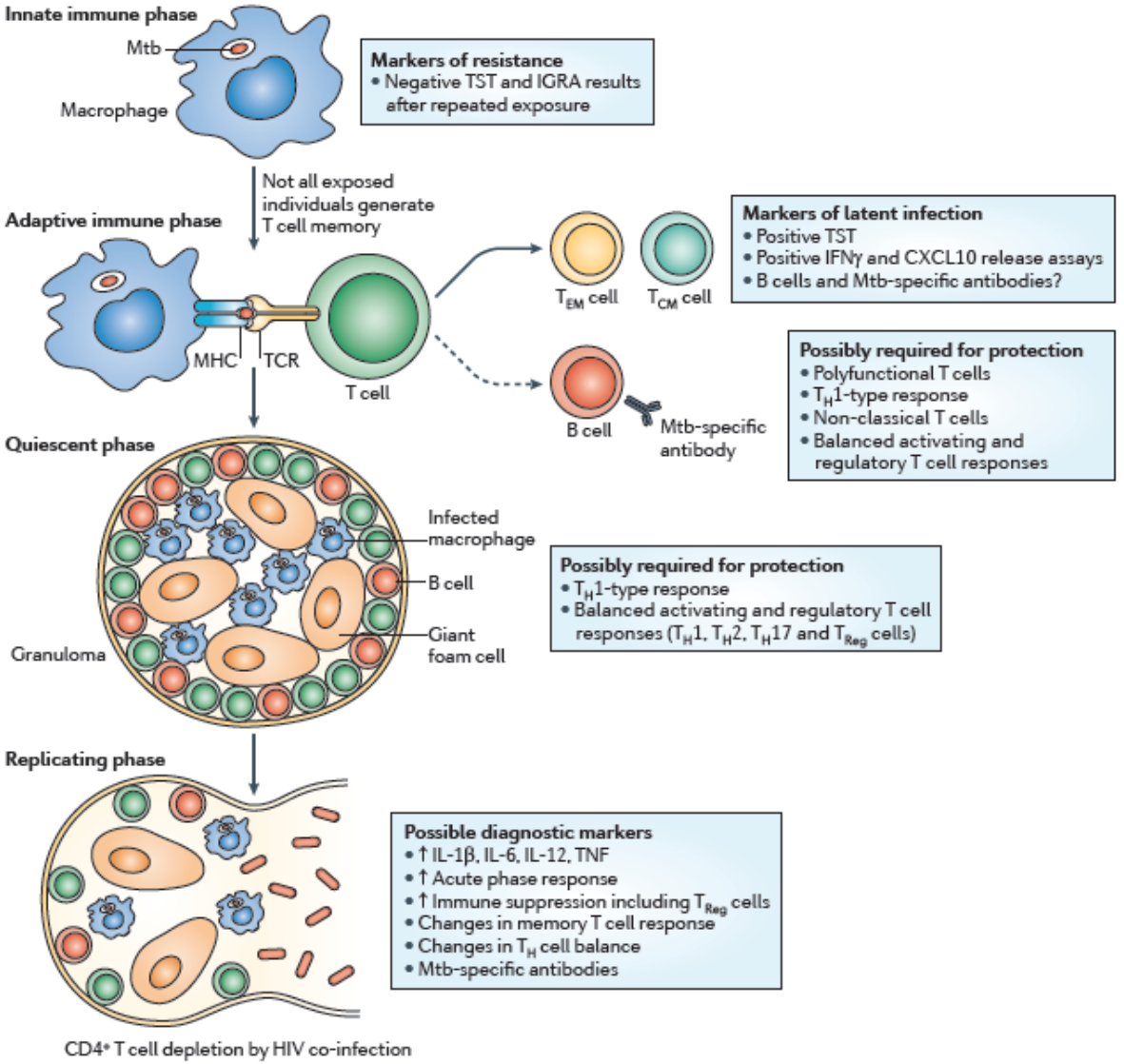


Figure 1. Immune mechanisms important in the maintenance of latent tuberculosis. The granuloma that forms in response to M tuberculosis *consists of macrophages, which can differentiate into epithelioid macrophages or multinucleate giant cells, CD4 and CD8 T cells, and B cells. The T cells produce interferon γ, which activates macrophages. CD8 T cells can lyse infected macrophages or kill intracellular bacteria. Tumour necrosis factor (TNF) is produced by macrophages and T cells. Dendritic cells are also present within the granuloma. A mature granuloma is surrounded by fibroblasts.* M tuberculosis *is present within the macrophages and also extracellularly if necrosis is present. On depletion of CD4 T cells (eg, during HIV infection), the granuloma does not function as well, production of interferon γ may decrease, and macrophages are less activated. As a result,* M tuberculosis *begins to multiply and cause reactivation of infection. In the case of TNF neutralisation, the cells within the granuloma are no longer as tightly clustered, perhaps owing to chemokine or adhesion-molecule dysregulation. In addition, the macrophages are not as activated. These defects lead to a disorganised granuloma that is less able to control infection and greater immunopathology.*

Latent *M. tuberculosis* infection
Latent infection with *M. tuberculosis* indicates the presence of live *M. tuberculosis* organisms in a human host who is asymptomatic. It is detected by demonstrating immune responsiveness of the host to *M. tuberculosis* antigens (using the tuberculin skin test or interferon-γ release assays). Latent infection can last a lifetime.

Active tuberculosis
The symptomatic disease caused by *M. tuberculosis* infection. Approximately 10% of infected individuals develop active disease in their lifetime owing to a loss of immune control over the pathogen. The disease manifests mainly in the lungs but can be extrapulmonary or disseminated.

Tuberculosis biomarker
An ideal tuberculosis biomarker should: differentiate between patients with active tuberculosis and individuals with latent *M. tuberculosis* infection; return to normal levels during treatment; reproducibly predict clinical outcomes (for example, cure, relapse risk or eradication of *M. tuberculosis* infection) in diverse patient populations; and predict vaccine efficacy and provide end points for clinical trials.



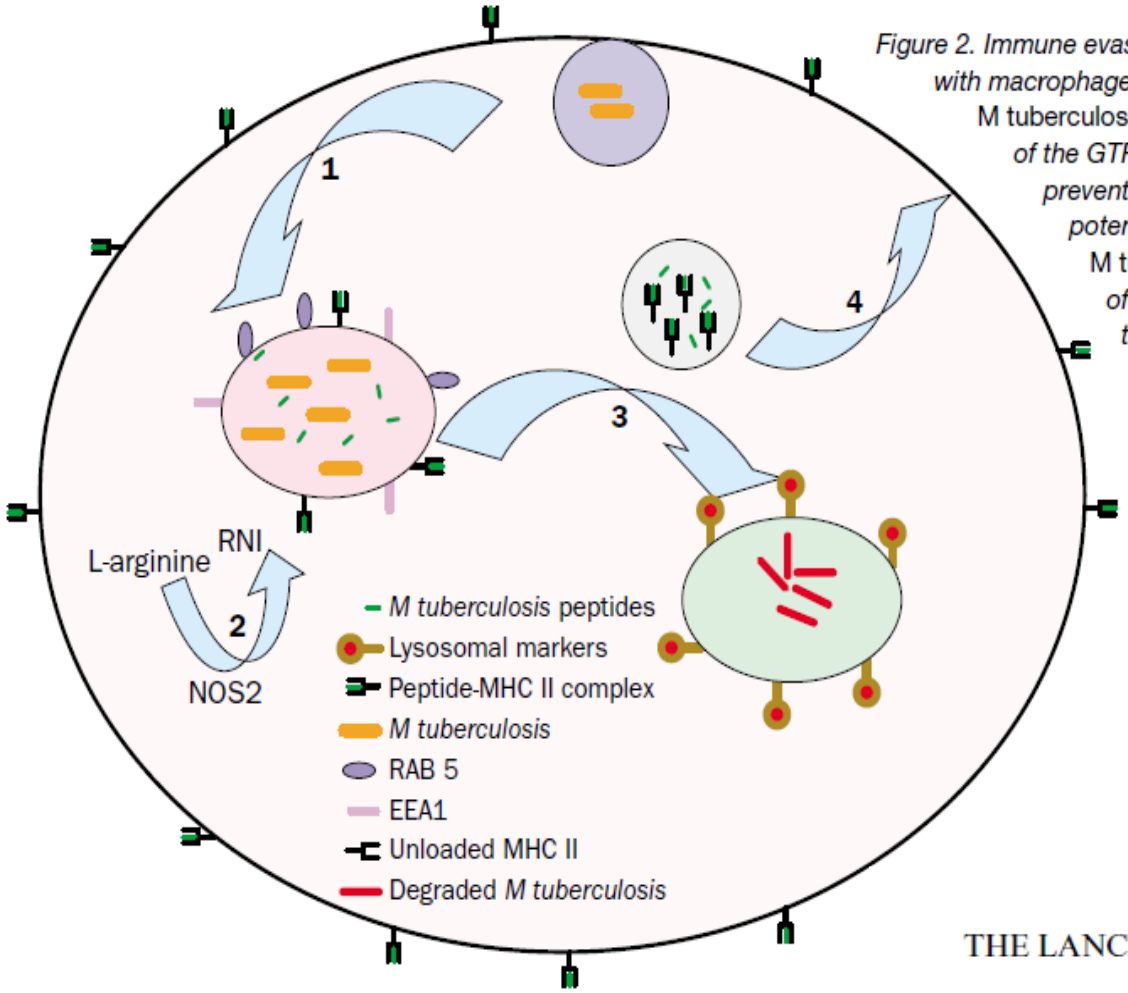


Figure 2. Immune evasion strategies of M tuberculosis*: interaction with macrophages. The maturation of phagosomes containing* M tuberculosis *apparently stops at a point close to the acquisition of the GTPase Rab5 (1). This arrest of phagosome biogenesis prevents fusion with lysosomal compartments (3) that have potent antimicrobial activities. Within the phagosome,* M tuberculosis *is subject to the antimycobacterial effect of reactive nitrogen intermediates (RNI) generated by the macrophage NOS2 (2). In addition,* M tuberculosis *can inhibit the MHC class II-dependent antigen presentation pathway (4). Thus,* M tuberculosis *can subvert various antimycobacterial functions of macrophages.*

THE LANCET Infectious Diseases Vol 3 September 2003

Other MTB evasion strategies include:

- **Manipulating cytokine responses (TLRs/DC-SIGN)**
- **Inhibiting macrophages apoptosis (probably neutrophils too)**
- **Transformation into a dormance stage (DosR)**

Vaccination Strategies (1)

1. **Pre-exposure vaccination** with superior BCG replacement to prevent tuberculosis in early childhood and to delay tuberculosis disease outbreak in adults.

2. **Pre-exposure boost** with subunit vaccine in children primed with BCG to prevent tuberculosis in early childhood and to delay tuberculosis disease outbreak in adults.

3. **Pre/Post-exposure boost** with subunit vaccine in adults who had been primed with BCG during early childhood to delay tuberculosis disease outbreak in adults.

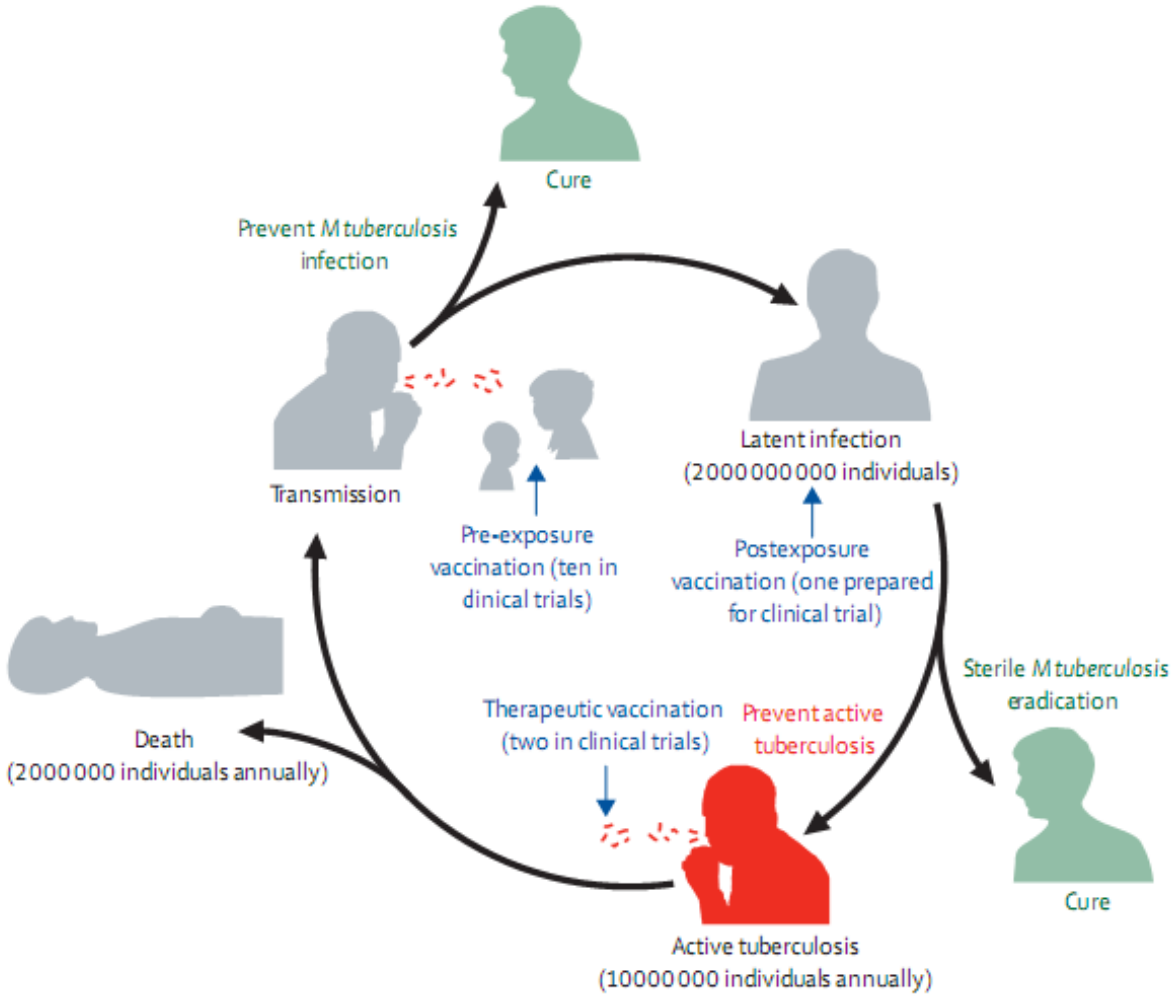
Vaccination Strategies (2)

4. Prime-boost vaccination with superior BCG replacement and subunit vaccine, to achieve **sterile eradication.**

5. Prime-boost vaccination in individuals with **latent infection** by prime with superior BCG replacement and subunit vaccine boost to prevent tuberculosis disease outbreak.

6. **Therapeutic vaccination** in adjunct to chemotherapy in patients with active tuberculosis.

Figure 1: **The life-cycle of *Mycobacterium tuberculosis* and different vaccination measures**
Vaccines in clinical trials are aimed at prevention of active tuberculosis. Future vaccines should aim to prevent or eradicate *M tuberculosis* infection.



Immunity 33, October 29, 2010

TB vaccines currently in clinical development

Vaccine (source, sponsor)	Description
BCG REPLACEMENT OR PRIMING VACCINE CANDIDATES	
rBCG30 (UCLA, Aeras)	Recombinant BCG overexpressing Ag85B
rBCGΔUreC:Hly; VPM-1002 (Max Planck Institute, VPM)	Recombinant BCG that perforates the endosome to enhance immunogenicity
AERAS-422 rBCG (Aeras)	Recombinant BCG that perforates the endosome and overexpresses Ag85A, Ag85B, and Rv3407
BOOSTING VACCINE CANDIDATES	
M72 + AS01 (GSK, Aeras)	Fusion protein, *Mtb* 39 + *Mtb* 32, plus AS01 adjuvant
MVA85A (Oxford, Emergent Biosolutions, Wellcome Trust, Aeras)	Nonreplicating vaccinia vector expressing Ag85A
AERAS-402/Ad35 (Crucell, Aeras)	Replication-deficient adenovirus vector expressing Ag85A, 85B, and 10.4
Ad Ag85A (McMaster University)	Adenovirus vector expressing Ag85A
Hybrid-1 + IC31 (Staten Serum Institut, Intercell)	Recombinant fusion protein Ag85B+ESAT-6 plus IC31 adjuvant
Hybrid-1 + CAF01 (Staten Serum Institut)	Recombinant fusion protein Ag85B+ESAT-6 plus CAF01 adjuvant
Hybrid-4/AERAS-404 + IC31(Staten Serum Institut, Sanofi-Pasteur, Intercell, Aeras)	Recombinant fusion protein Ag85B+TB10.4 plus IC31 adjuvant

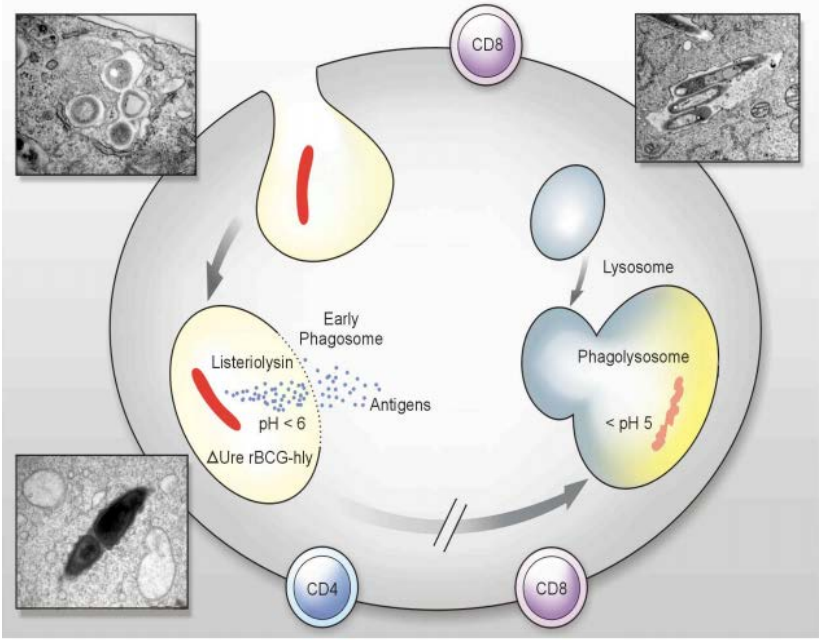
Live r-BCG prime vaccines designed to replace BCG

1- rBCG30:

- Genetically modified BCG such that it overexpresses an early MTB target for the host immune response (antigen 85B).
- In Phase I trial rBCG30 was well tolerated and had a comparable safety profile to non recombinant BCG.
- Antigen 85B-specific T-cell proliferation and IFN-γ ELISpot responses were enhanced and antigen specific CD4+ and CD8+ effector T-cell expansion was demonstrated.
- The antigen 85B-specific T cells induced were also capable of inhibiting the growth of intracellular mycobacteria.
- But, the vaccine is not being further developed currently.

2- VPM1002 rBCGΔureC::Hly

- Expresses listeriolysin (Hly) derived from Listeria monocytogenes and enables BCG to escape from the endosome.
- Made urease-C-deficient to provide the optimal pH for Hly activity.
- PEST sequence allows Hly degradation in the cytosol.
- A Phase I clinical trial evaluating the safety and immunogenicity of this vaccine in healthy male subjects has been completed
- The vaccine is currently in a Phase II trial in newborn infants.



3- rBCG::pfo/AERAS 422

- Expresses perfringolysin O (Pfo) as an endosome escape mechanism.
- Overexpresses immunodominant and protective M. tuberculosis antigens, 85A, 85B and Rv3407.
- A Phase I clinical trial in healthy human subjects had to be terminated due to side effects.
- Indeed, Pfo lacks the PEST sequence and hence is not degraded in the cytosol where it could damage its host cell.

Subunit vaccines designed to enhance BCG effectiveness

- Regimens would retain BCG vaccination of neonates
- Involve the delivery of immunodominant mycobacterial antigens to boost the immune system, using:

1. Viral vectored vaccines
2. Protein – adjuvant vaccines

MVA85A

- MVA (modified vaccinia virus Ankara) as a delivery system for the mycobacterial antigen 85A.
- Evaluated in a series of Phase I clinical trials in healthy adults in the UK since 2002, including BCG-vaccinated subjects and subjects with LTBI.
- Phase I and IIa clinical trials in target populations in South Africa, The Gambia and Senegal.
- Has been safely administered to high-risk target populations, namely HIV-infected adults, subjects co-infected with HIV and M. tuberculosis and infants.

Results of MVA85 so far…

- High frequencies of antigen-specific IFNγ–producing polyfunctional CD4+ T cells are induced.
- Expansion of a memory population.
- Frequency of antigen-specific cells remains. significantly higher than baseline for at least 1 year after vaccination.
- Antigen-specific, IFNγ-producing CD8+ T cells have also been detected.
- A Phase IIb efficacy trial in BCG-vaccinated South African infants is now underway.

MVA85 and EPI Vaccines

- **Immunogenicity of MVA85A is reduced by coadministration with EPI Vaccines in a randomized controlled trial in Gambian infants** Sci. Transl. Med. 2011, 3(88).
- Coadministration of MVA85A with EPI vaccines was associated with a significant reduction in MVA85A immunogenicity, but did not affect humoral responses to the EPI vaccines.
- Suggest that modifications to the standard EPI schedule may be required to incorporate a new generation of T cell–inducing vaccines.

AERAS-402/Crucell Ad35

- Replication-deficient adenovirus (Ad) type 35 as vector
- Expressing a fusion protein of mycobacterial antigens 85A, 85B and TB10.4
- IM administration of was well tolerated and induced polyfunctional CD4+ T cells and IFNγ–producing CD8+ T cells in response to antigens stimulation.
- A Phase II trial in South Africa is recruiting HIV-infected, BCG-vaccinated adults and assessing the safety (including effect on CD4 count) and immunogenicity.
- In Kenya, a Phase I and II safety, immunogenicity and efficacy trial in BCG-vaccinated, HIV-uninfected infants is ongoing.

Ad5Ag85A

- Intranasal but not IM administration afforded better protection against M. tuberculosis aerosol challenge than cutaneous BCG and enhanced protection when given as a boost to BCG in both BALB/c mice and guinea pigs.
- A safety and immunogenicity Phase I trial in humans is ongoing in Canada in healthy BCG-vaccinated and unvaccinated subjects.
- Pre-existing antibodies against adenoviruses and notably Ad5 could impair efficacy, simian Ad are an alternative for viral-vectored vaccine candidates of the next generation.

Box 3. Adjuvants used in TB vaccines

- IC-31 is a mixture of a cationic antimicrobial peptide and an oligodeoxynucleotide ligand for Toll-like receptor (TLR)9, which facilitates antigen uptake and sustenance and stimulates DCs [82]. This adjuvant primarily stimulates CD4 Th1 cells, but also CD8 CTLs.
- $AS01_E$ is a liposomal formulation composed of the TLR4 ligand, monophosphoryl lipid A, and the surface-active compound QS, to stimulate CD4 Th1 cells, probably CD4 Th17 cells, and CD8 CTLs [36].
- CAF01 adjuvant is a liposomal formulation containing dimethyl-dioctadecyl ammonium bromide and trehalose 6,6- dibehenate, which delays antigen release, and stimulates CD4 Th1, Th2, and Th17 cells, as well as CD8 CTLs [83].

http://dx.doi.org/10.1016/j.it.2012.03.004 Trends in Immunology xx (2012) 1–7

M72

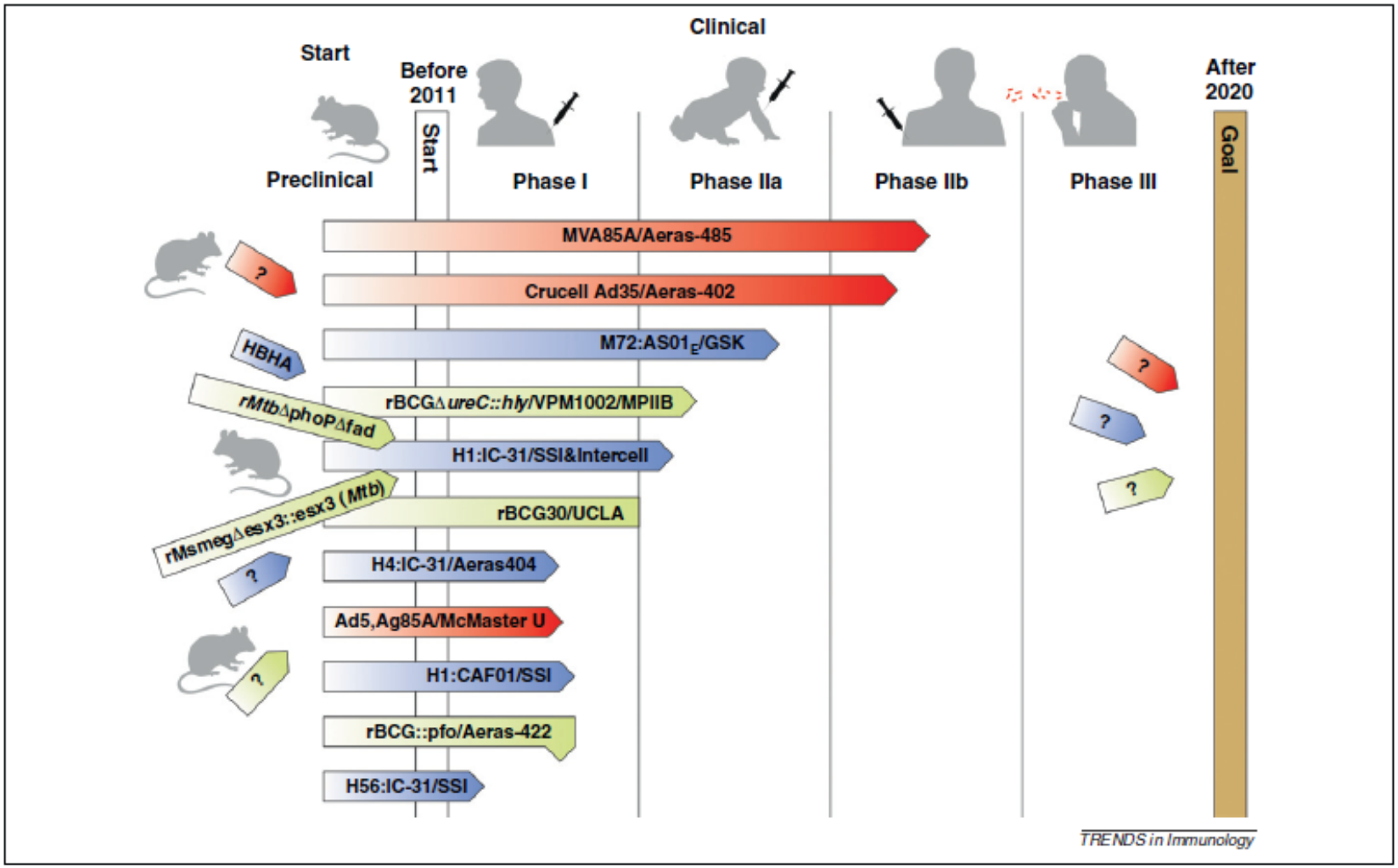
- Fusion of a protein from the PPE family (Rv1196) and an inactive serine protease (Rv0125) in AS series adjuvants.
- Now being brought forward in a formulation with AS01E.
- Well tolerated in a Phase I trial of PPD-healthy adult subjects in the USA and induced antigen-specif ic IFN-γ and IL-2 production and CD4+ T cells.
- Phase IIa trials in TST-positive healthy adults in a TB-endemic area (South Africa) and of different formulations in the Philippines have also been completed.

Hybrids

- H1: Fusion protein of antigens 85B and ESAT-6 adjuvanted with IC-31, in a Phase I trial in PPD-negative healthy adults was associated with antigen-specific T-cell responses maintained for 2 years. Now also adjuvanted with CAF01 in PhaseI trial.

- H4: ESAT-6 was changed to TB10.4 to avoid cross-reactivity in IGRA used for diagnosis of MTB infection (with or without active disease). Indeed, false positive responses are observed in H1-vaccinated individuals. Currently in ongoing Phase I trial.

- H56 : In addition to H1 antigens contains an antigen predominantly expressed by dormant MTB (Rv2660). This vaccine is considered for pre- and post-exposure administration.



http://dx.doi.org/10.1016/j.it.2012.03.004 Trends in Immunology xx (2012) 1–7

Therapeutic vaccines in clinical trials

- **Mycobacterium vaccae** : 1 dose not very effective. 5 doses in BCG-vaccinated, HIV-infected patients in Tanzania demonstrated significant protection against the secondary end point of definite (culture positive) TB, although not against the primary end point of disseminated (bacteremic) disease or against the other secondary end point, probable TB
- **RUTI® (Archivel Farma):** detoxified liposomal cellular fragments of M. tuberculosis bacilli. In a double-blind Phase I RCT in BCG-naive healthy men in Spain, RUTI was well tolerated and associated with modestly enhanced responses to PPD and mycobacterial antigens, including ESAT-6 and 85B .

The three phases of clinical trials for TB vaccines

Clinical trials for TB vaccines follow general guidelines with specific modifications (http://clinicaltrials.gov/).

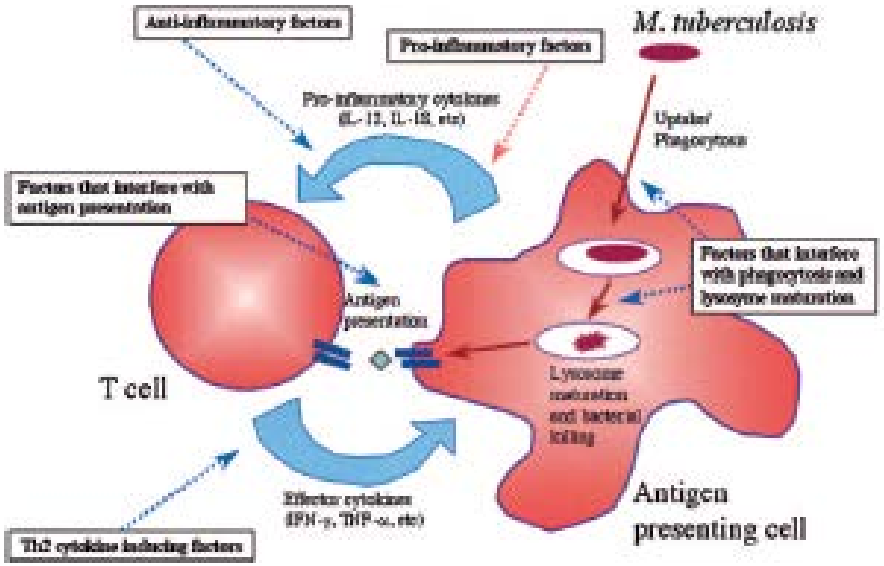
Phase I: Safety and immunogenicity testing in small study groups (10 or more individuals per group). Typically, Phase I trials are first performed in the region where the vaccine candidate was developed and then repeated under similar conditions in a highly endemic area. Eleven TB vaccine candidates have passed Phase I clinical trials.

Phase II: Assessment in larger groups (100 or more individuals per group) of optimal dosage and route of administration; efficacy is based on immune parameters ideally via a biomarker or biosignature as correlate of protection. Currently, no correlate of protection against TB exists that can reliably predict TB disease outbreak. Four vaccine candidates have entered or passed Phase II clinical trials.

Phase III: Assessment of vaccine efficacy against natural infection monitoring of safety in a highly endemic area. Prophylactic TB vaccine trials will need to involve 20,000–50,000 participants and will last several years because even in highly endemic areas, incidences rarely exceed 300/100,000 adults, and incubation time often lasts for years. Moreover, it will be necessary to perform several phase III trials in different regions.

Box 1. The Three Phases of Clinical Trials for TB Vaccines

Immunity *33*, October 29, 2010


Anti-inflammatory factors
Pro-inflammatory factors
M. tuberculosis
Pro-inflammatory cytokines
(IL-12, IL-18, etc)
Uptake/
Phagocytosis
Factors that interfere with
antigen presentation
Factors that interfere
with phagocytosis and
lysosyme maturation
Antigen
presentation
T cell
Lysosome
maturation
and bacterial
killing
Antigen
presenting cell
Effector cytokines
(IFN-γ, TNF-α, etc)
Th2 cytokine inducing factors

TB vaccine candidates in the clinic.

Vaccine	Source	Stage	Description
Priming vaccines			
BCG30	UCLA/Aeras	Phase 1 US	rBCG with plasmid overexpressing Ag85A
VPM-X	Max Plank/VPM/TBVI	Phase 1 Europe	rBCG with chromosomal expression of listeriolysin
AERAS-rBCG	Aeras	Phase I (2009) US	rBCG with chromosomal expression of perfringolysin and Ag85A, Ag86B, Rv3427, rpfA, rpfB, rpfC, and Dos R regulated proteins
Viral vectored booster vaccines			
MVA85A/AERAS-485	Oxford/Isis/Aeras/Emergent	Phase IIb Europe, Africa	Recombinant modified vaccinia vectored vaccine expressing Ag85A
AERAS-402/ Crucell Ad35	Crucell/Aeras	Phase I and IIa US	Recombinant adenovirus 35 expressing antigens Ag 85A, Ag 85B, and Mtb10.4
Protein booster vaccines			
GSK M72	GSK/Aeras	Phase I and IIa Europe, Africa	Fusion molecule comprised a protein from the PPE family Rv1196 and an inactive serine protease Rv0125 with AS01 adjuvant
Hybrid I	SSI/Intercell/TBVI	Phase I and IIa Europe, Africa	Fusion molecule comprised Ag85B and AgESAT-6 with adjuvant IC31
HyVac 4/AERAS-404	Sanofi Pasteur/SSI/Intercell/Aeras	Phase I Europe, Africa	Fusion molecule comprised Ag85B and Mtb10.4 with adjuvant IC31

Box 1 Prime-boost strategies for preventing different forms of TB disease

Immunization strategy	BCG replacement/prime	Boost	Latency vaccine/immunotherapy
Prevention of clinical pathology	Meningeal/miliary TB	Pulmonary TB	Reactivation TB (pulmonary TB)
TB vaccine candidates	Live rBCG expressing *Mtb* antigens	Viral vectored, for example, vaccinia, adenovirus-35	Live or acellular vaccines containing latent stage antigens, for example, Rv3427, DosR regulated antigens, HBHA
	Live rBCG with endosome pertubation properties	Proteins with adjuvant, for example, 85A/ESAT-6 IC31, 85A/10.4 IC31, 72f AS01	
	Live attenuated *Mtb*		

rBCG—recombinant BCG; HBHA—heparin-binding hemagglutinin.

MVA85A in HIV+ adults

- A Phase I study evaluating the safety and immunogenicity of MVA85A, a candidate TB vaccine, in HIV-infected adults.
  BMJ Open 2011;1:e000223. doi:10.1136/ bmjopen-2011-000223
- MVA85A was safe in subjects with HIV infection,
- No clinically significant vaccine-related changes in CD4 count or HIV RNA load in any subjects,
- Both doses of MVA85A induced an antigen-specific IFN- response that was durable for 24 weeks,
- The functional quality of the vaccine-induced T cell response in HIV-infected subjects was remarkably comparable with that observed in healthy HIV-uninfected controls

MVA 85A in Infants

- Dose-Finding Study of the Novel Tuberculosis Vaccine, MVA85A, in Healthy BCG-Vaccinated Infants. JID 2011;203:1832–43
- Infants aged 5–12 months were vaccinated intradermally with either of 3 escalating dose schedule of MVA85A, or placebo.
- MVA85A induced potent, durable T-cell responses, which exceeded prevaccination responses up to 168 days after vaccination.
- Conclusions. MVA85A was safe and induced robust, polyfunctional, durable CD4 and CD8 T-cell responses in infants. These data support efficacy evaluation of MVA85A to prevent tuberculosis in infancy.

Protein–adjuvant vaccines

- Adjuvants used may be – aluminium based compounds or immunopotentiating agents eg, TLR ligands, saponins, cytokines and bacterial toxins

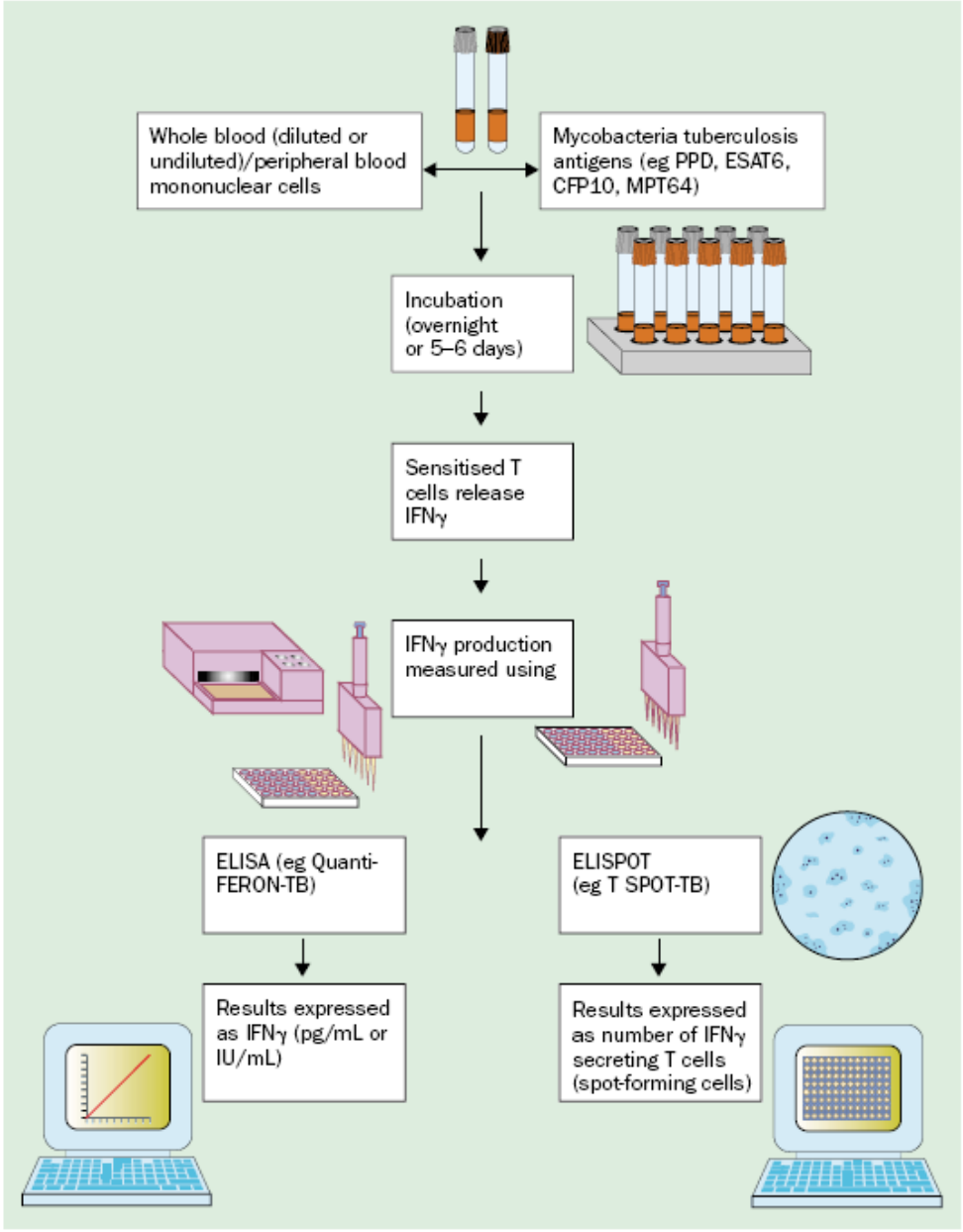
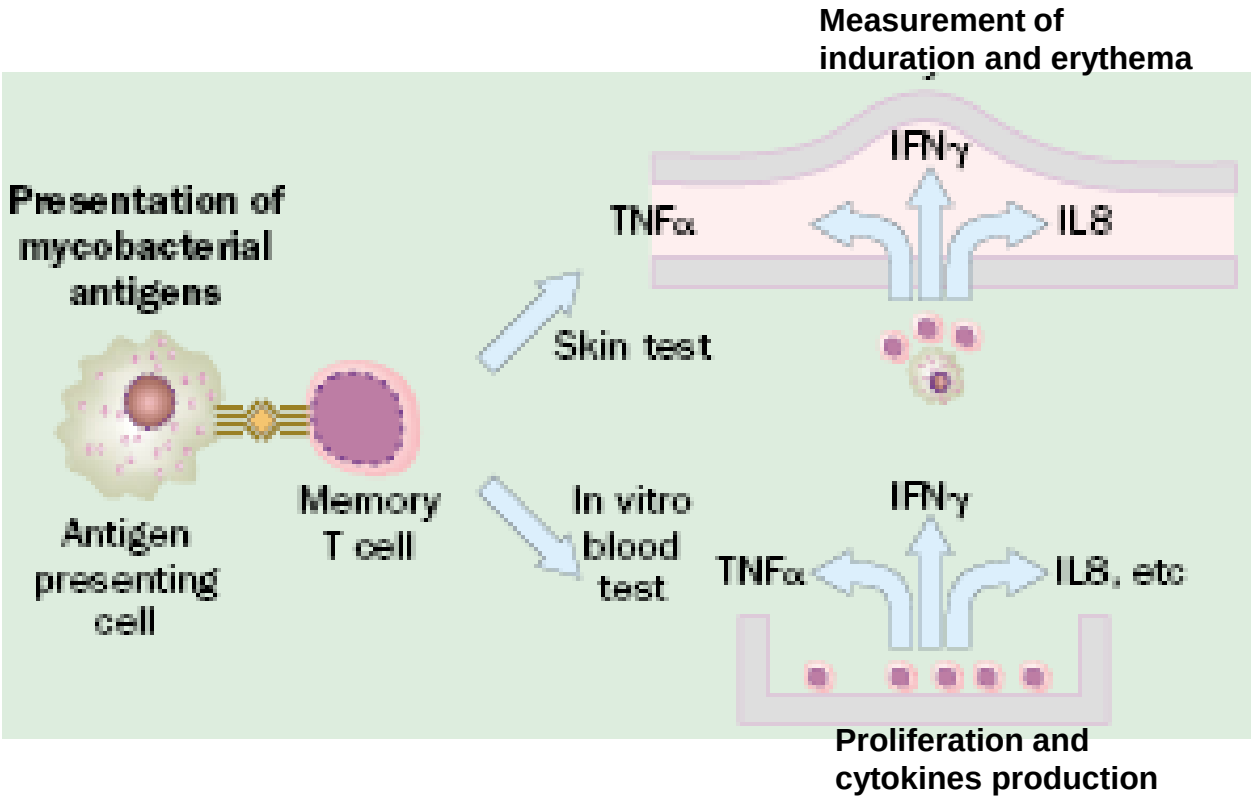
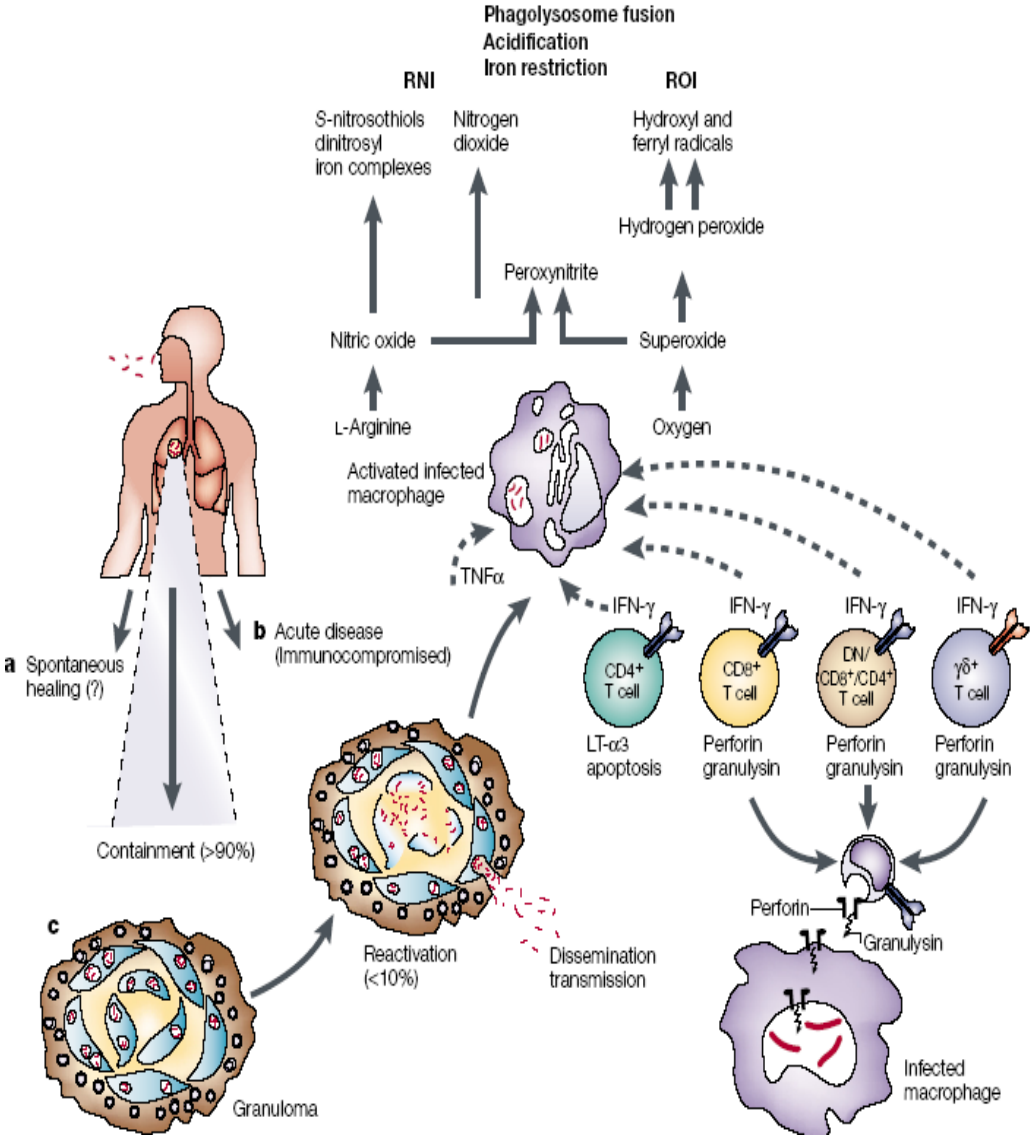
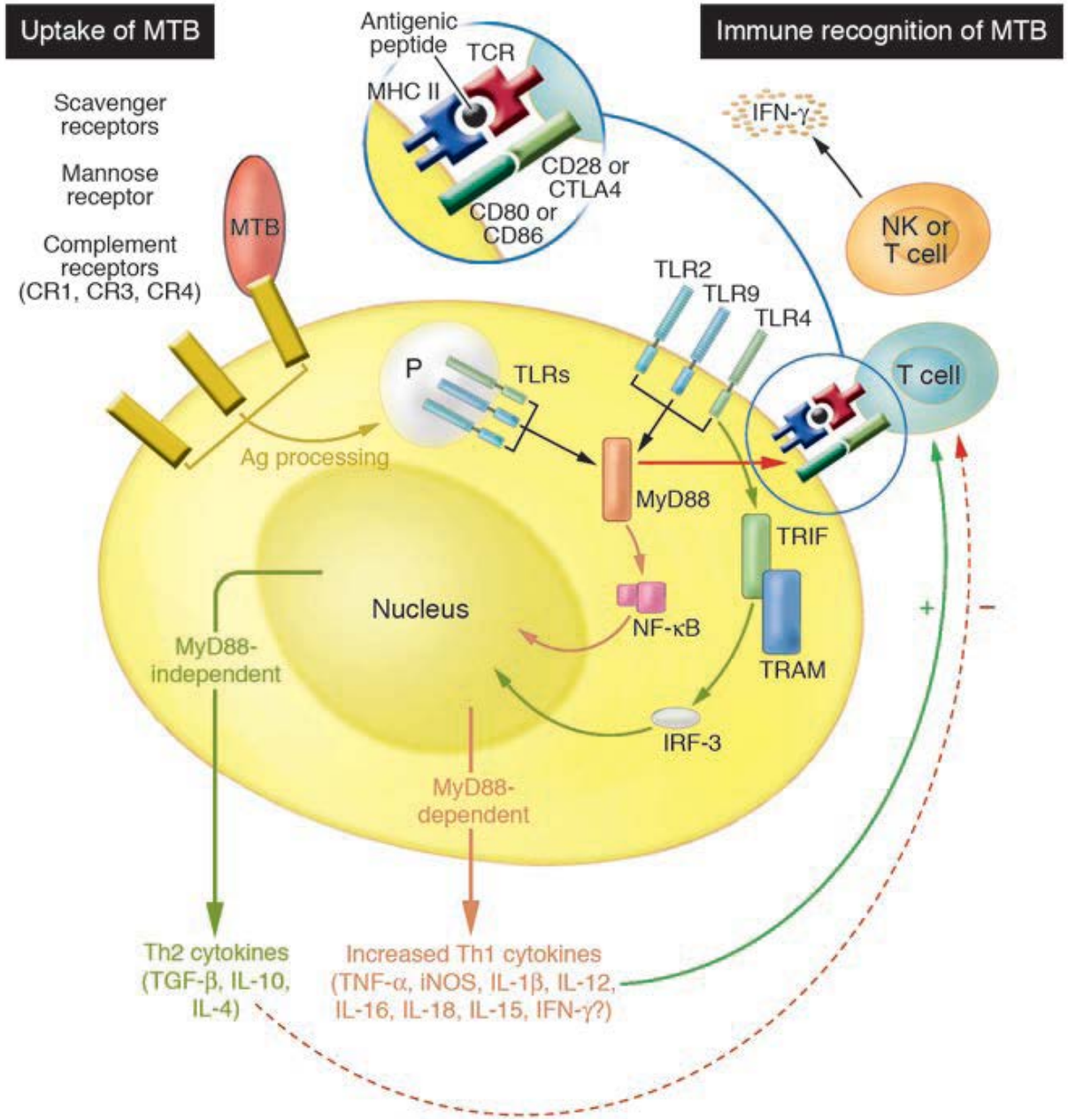


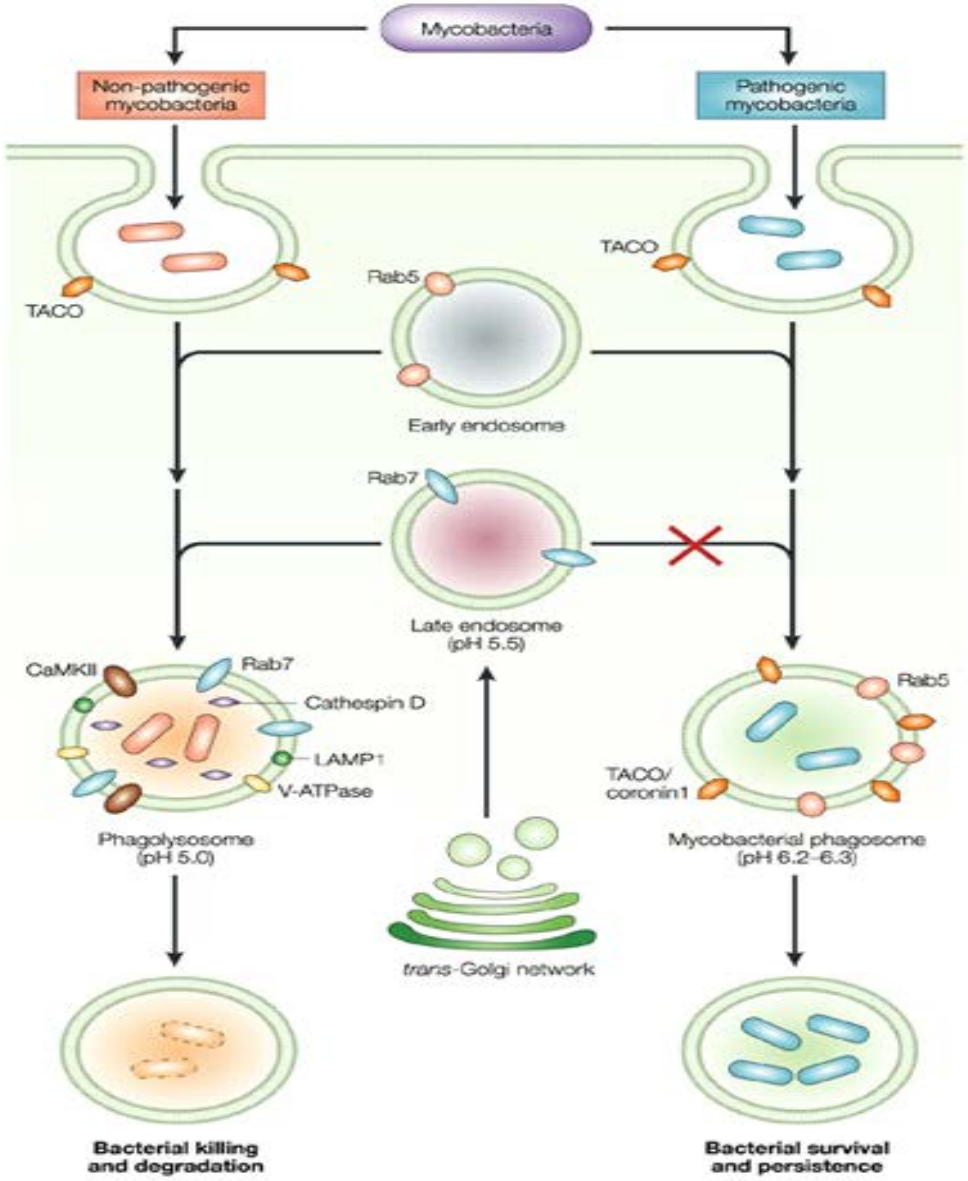
Figure 2. Overview of the interferon-γ (IFN-γ) assay technology.

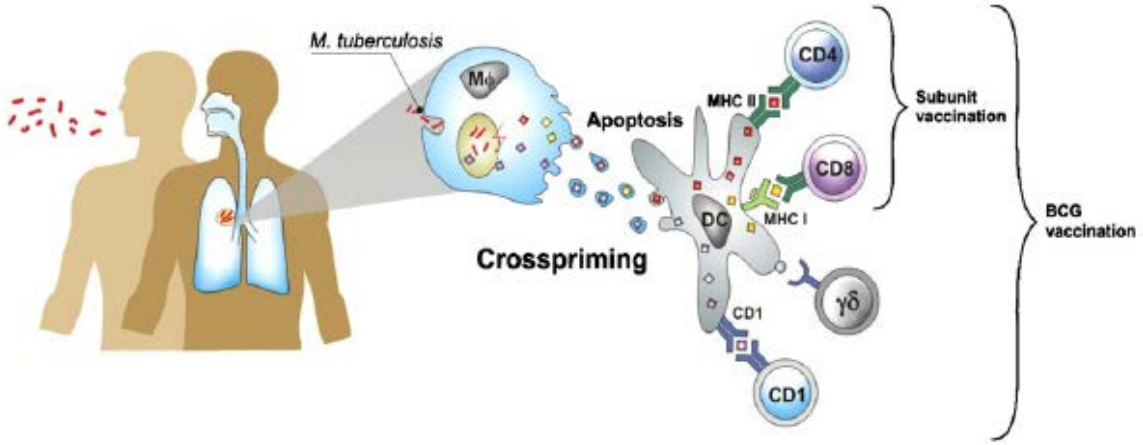


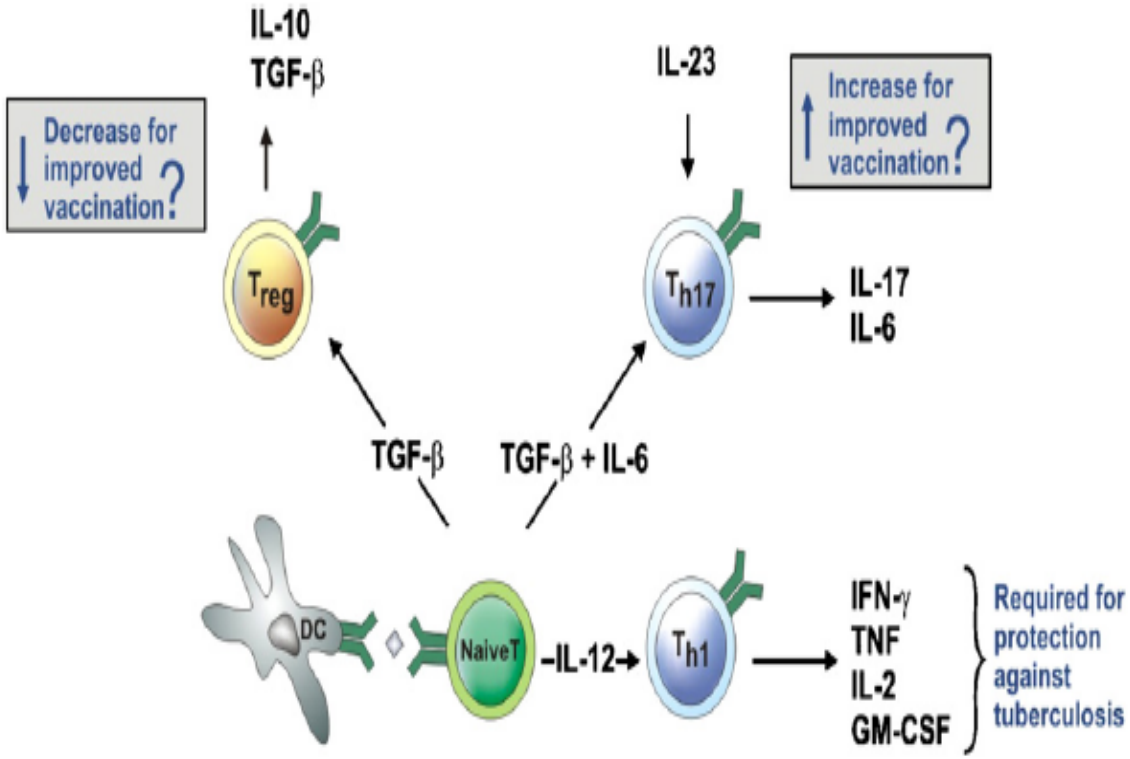
Biological basis of the tuberculin skin test, proliferation and IFNγ assays.








M. tuberculosis
Mϕ
Apoptosis
Crosspriming
DC
CD4
MHC II
CD8
MHC I
CD1
γδ
CD1
Subunit vaccination
BCG vaccination


IL-10
TGF-β
Decrease for improved vaccination?
IL-23
Increase for improved vaccination?
Treg
Th17
IL-17
IL-6
TGF-β
TGF-β + IL-6
DC
NaiveT
–IL-12→
Th1
IFN-γ
TNF
IL-2
GM-CSF
Required for protection against tuberculosis