



Network di Microbiologia e Virologia del Nord Est

Incontro di Aggiornamento

**INTERFERON GAMMA RELEASE ASSAYS (IGRAs) NELLA
DIAGNOSI E MONITORAGGIO DELLE MALATTIE INFETTIVE**

19 aprile 2013

Sala Ferrari Incontri - Cantine Ferrari
Via del Ponte di Ravina, 15
38123 Trento



La valutazione del rischio tuberculare in pazienti critici

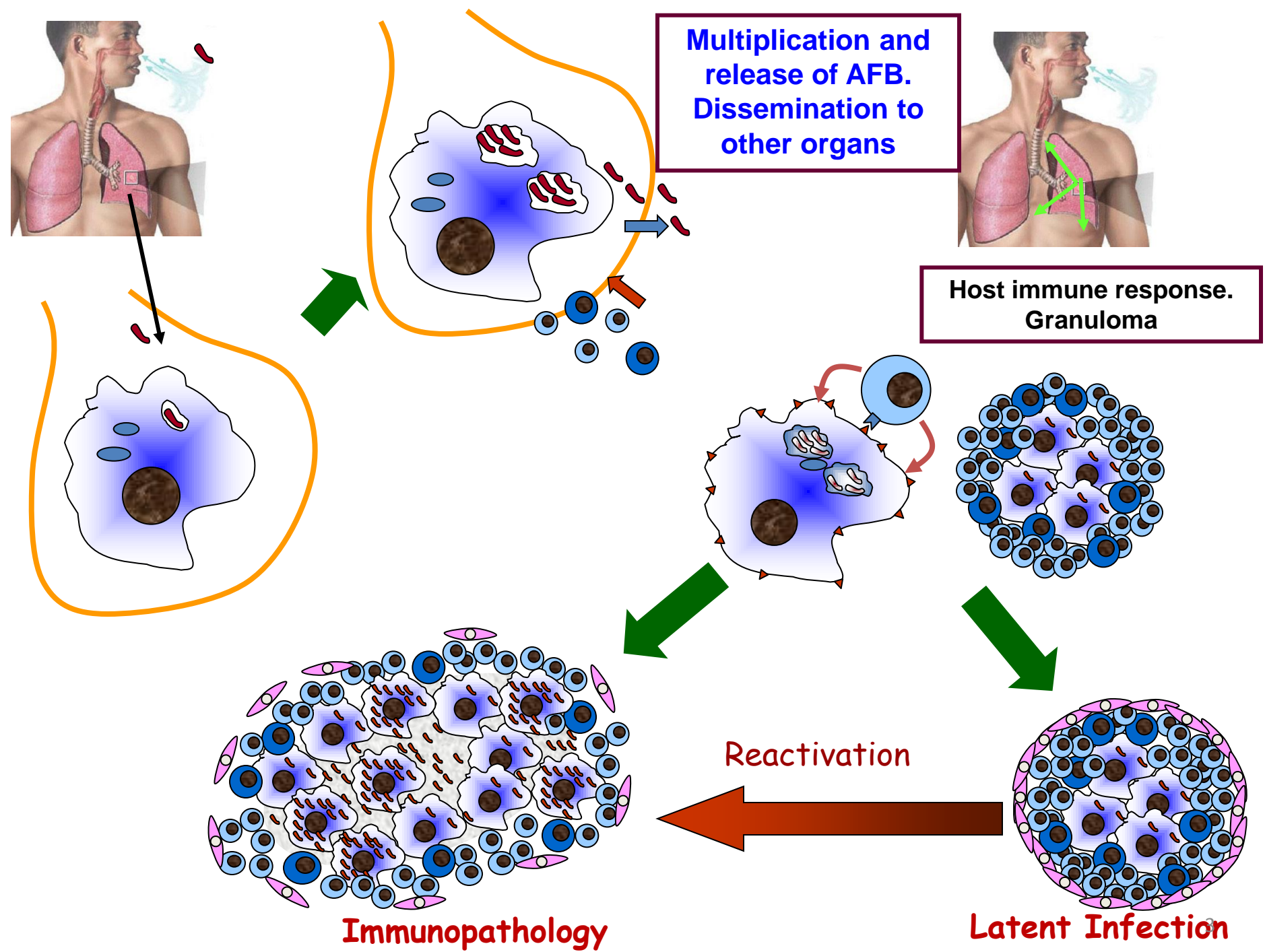
Giovanni Delogu

Istituto di Microbiologia

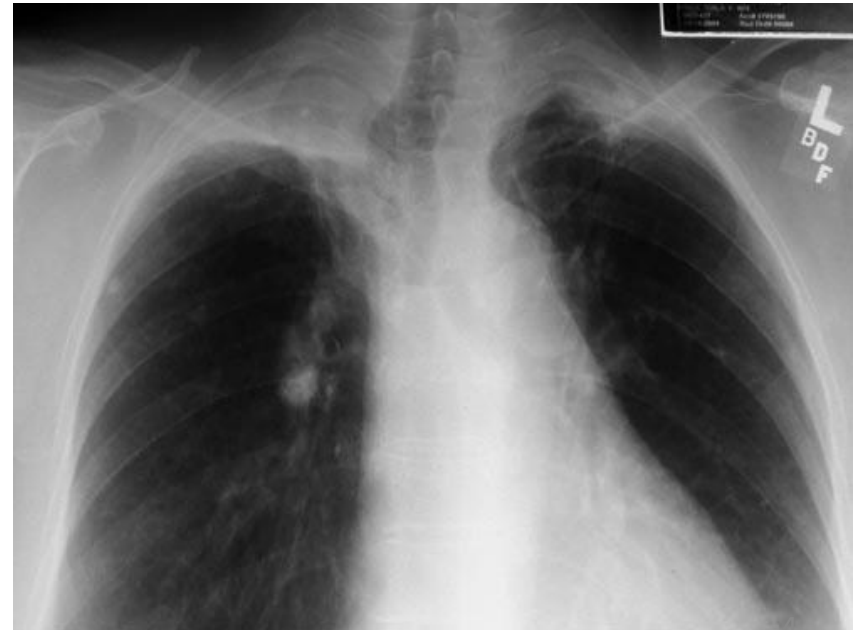
Università Cattolica del Sacro Cuore, Roma

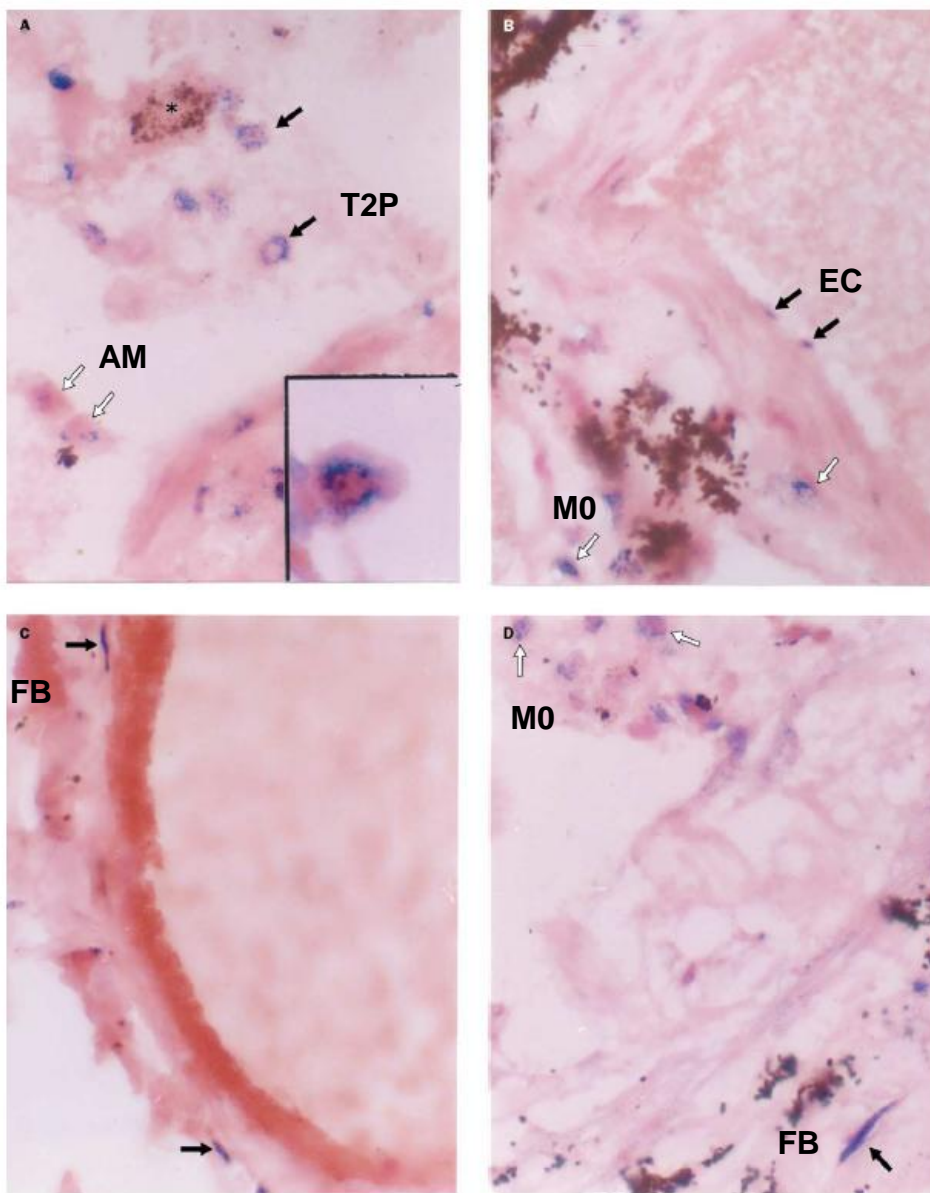
Outline

- ✓ **Dinamica della risposta immunitaria in corso di infezione latente;**
- ✓ **Valutazione del rischio in pazienti sottoposti a terapie immunosoppressiva;**
- ✓ **Valutazione del rischio nella popolazione pediatrica;**



- **The Ghon complex was considered the “sanctuary” of Mtb and destruction of this old lesion would result in bacteria replication and spread, tissue damage and TB reactivation.**
- **Encapsulated lesions isolated from LTBI subjects were shown to be microbiologically sterile (Mtb could not be cultured) and a higher bacterial viability was observed in fibrotic and caseous lesions or from tissue homogenates from unaffected portions of the lung (Bishai WR Lancet 2000);**





Representative micrographs of the cellular localisation of mycobacterial DNA revealed by in-situ PCR in lung tissue from a single donor without tuberculous disease

A=Alveolar macrophages showing positive (blue) mycobacterial DNA labelling (black arrows). These cells are intermixed with other macrophages containing black phagocytosed carbon material (*). Positive round cells on the alveolar wall correspond to type II pneumocytes (white arrows and insert). B=Venal endothelial cells (black arrows) and macrophages (white arrows) surrounding carbon deposits showing mycobacterial DNA labelling. C=Positive fibroblasts (black arrows) located in the adventitial layer from an intermediate-sized vein. D=A fibroblast (black arrow) and macrophages (white arrows) in fibrotic subpleural bands show strong mycobacterial DNA labelling. Original magnification $\times 400$ (Insert $\times 100$).

- *M. tuberculosis* can persist intracellularly in lung tissue without histological evidence of tuberculous lesions.
- *M. tuberculosis* DNA is situated not only in macrophages but also in other non-professional phagocytic cells.
- These findings contradict the dominant view that latent organisms exist in old classic tuberculous lesions, and have important implications for strategies aimed at the elimination of latent and persistent bacilli.

Hernandez-Pando R *et al.*, Lancet 2000

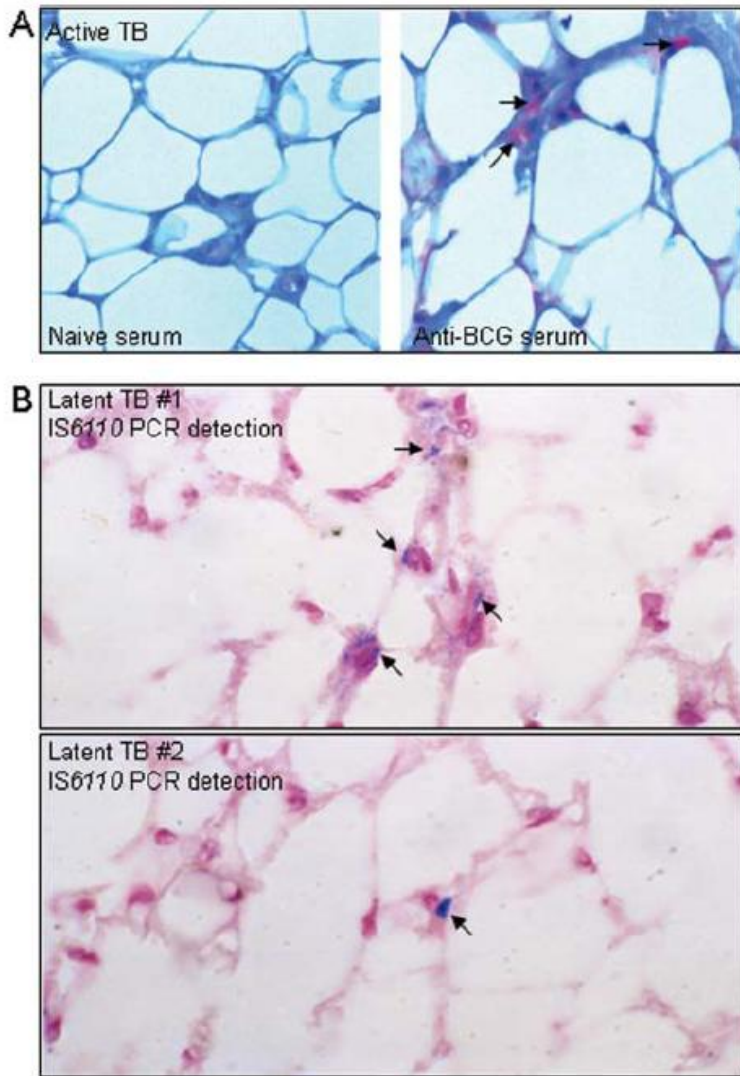
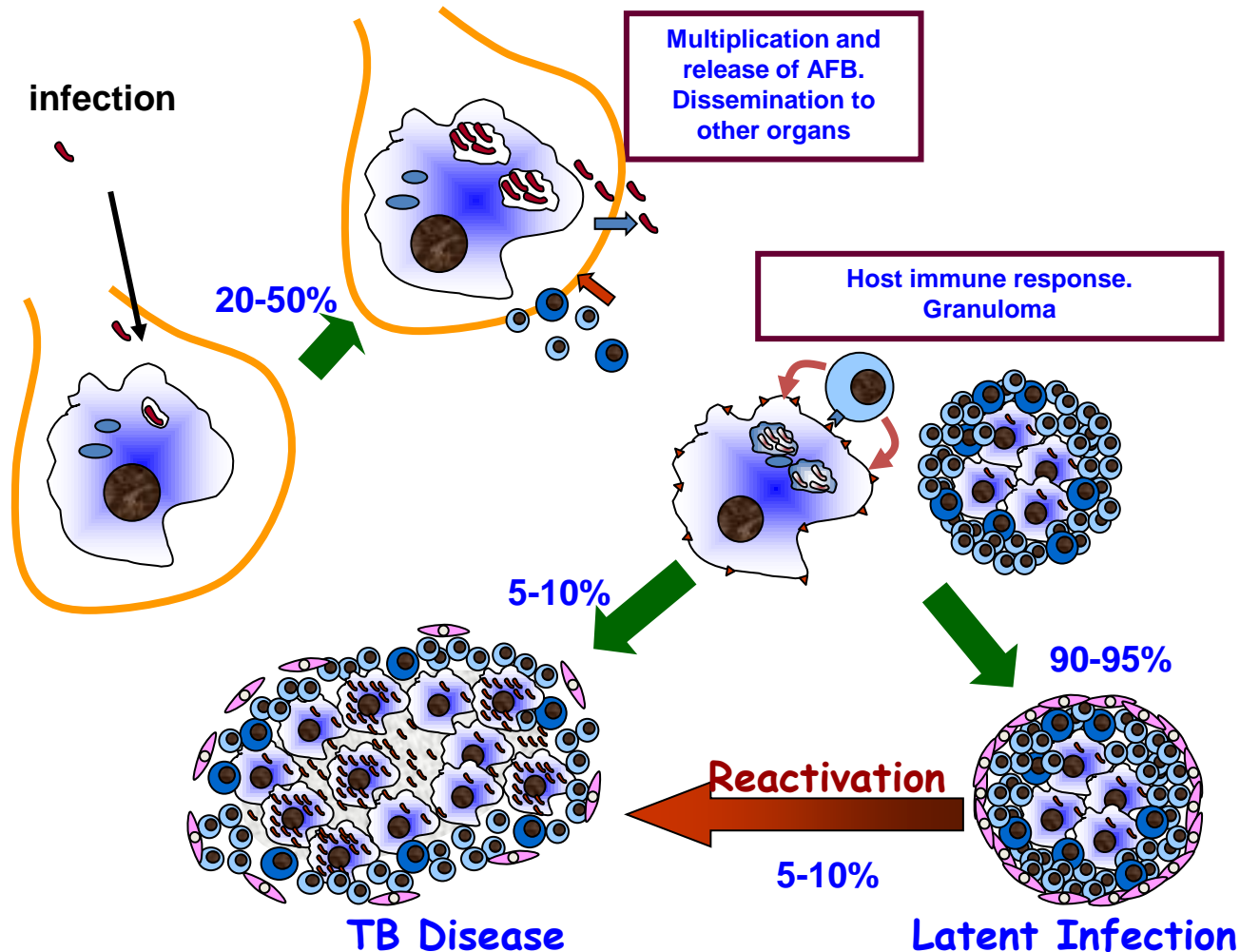


Figure 3. Detection of *M. tuberculosis* in adipose tissue from individuals with latent or active TB. **A.** Perinodal adipose tissue was taken from biopsy of the mediastinal lymph node from a patient with active TB. Bacilli were immuno-detected using an anti-BCG rabbit hyperimmune serum (right panel). As a control, a serial section was stained with serum from a naïve animal (left panel). **B.** Perinodal adipose tissue was taken at autopsy from two individuals (upper and lower panels) with no clinical sign of pulmonary TB. *In situ* PCR was used to detect IS6110. In A&B, arrows indicate positive signals.
doi:10.1371/journal.pone.0000043.g003

“Given the abundance and the wide distribution of the adipose tissue throughout the body, our results suggest that this tissue, among others, might constitute a vast reservoir where the tubercle bacillus could persist for long periods of time, and avoid both killing by antimicrobials and recognition by the host immune system.”

Evidences indicate that during latent infection, the tubercle bacilli resides in many different tissues, that are not associated with the site of primary infection.



What are the metabolic states during infection?

- Hypoxic, nutrient starvation, NO, CO;
- Dormancy (*dos regulon*);
- Resuscitation (*rpf*);

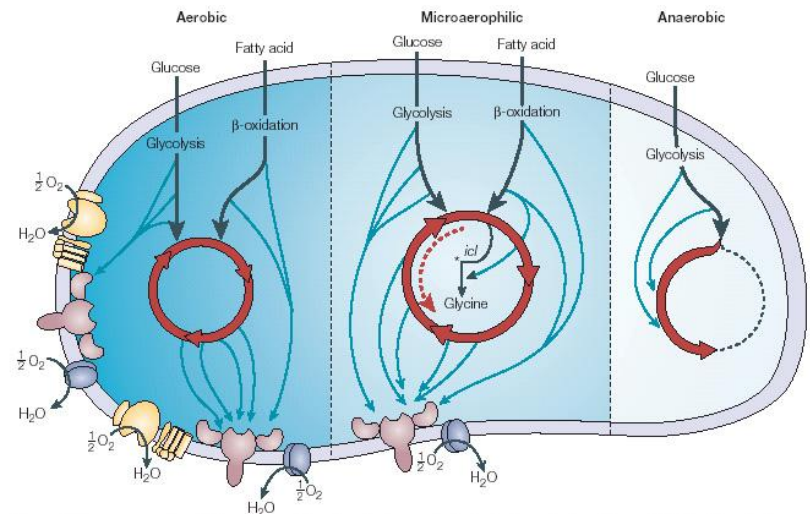


Figure 4 | The proposed route for regeneration of reducing equivalents is dependent on the availability of terminal electron acceptors. Under aerobic conditions, reducing equivalents that are generated during glycolysis, the Krebs cycle and β -oxidation are reoxidized by the electron-transport chain, terminating in cytochrome *c* and cytochrome *bd* oxidases. Under microaerophilic environments, these reducing equivalents are reoxidized by respiration and mainly use the high oxygen-affinity cytochrome *bd* oxidase, although reductive amination of glyoxylate (*) would reoxidize half of the reducing equivalents produced during β -oxidation. Under anaerobic conditions, the proposed route for reoxidation of reduced cofactors produced during glycolysis would be through the reductive branch of the Krebs cycle or by lactate dehydrogenase (not shown). Reducing equivalents could also be reoxidized in the presence of nitrate by the respiratory nitrate reductase (not shown). Isocitrate lyase, which is encoded by the *iscI* gene, is a central enzyme in the glyoxylate shunt pathway.

Drug	Metabolically active bacilli	Dormant bacilli
Isoniazid	+++	-
Rifampicin	+++	+
Metronidazole	-	++
Pyrazinamide	+	+++

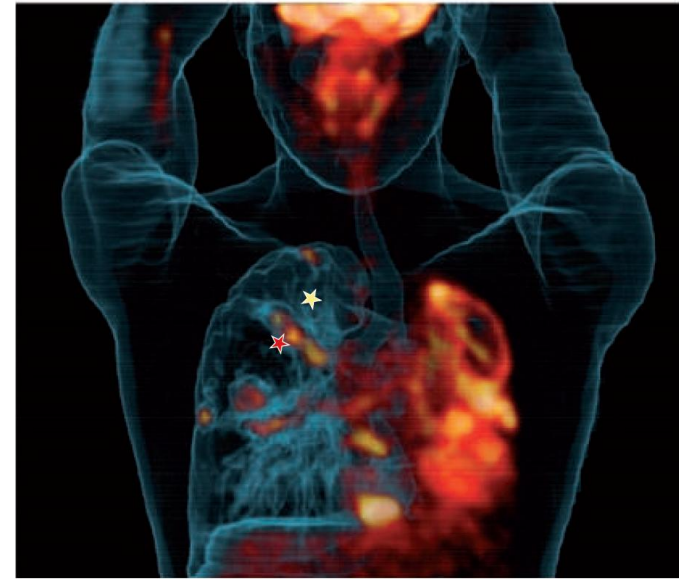
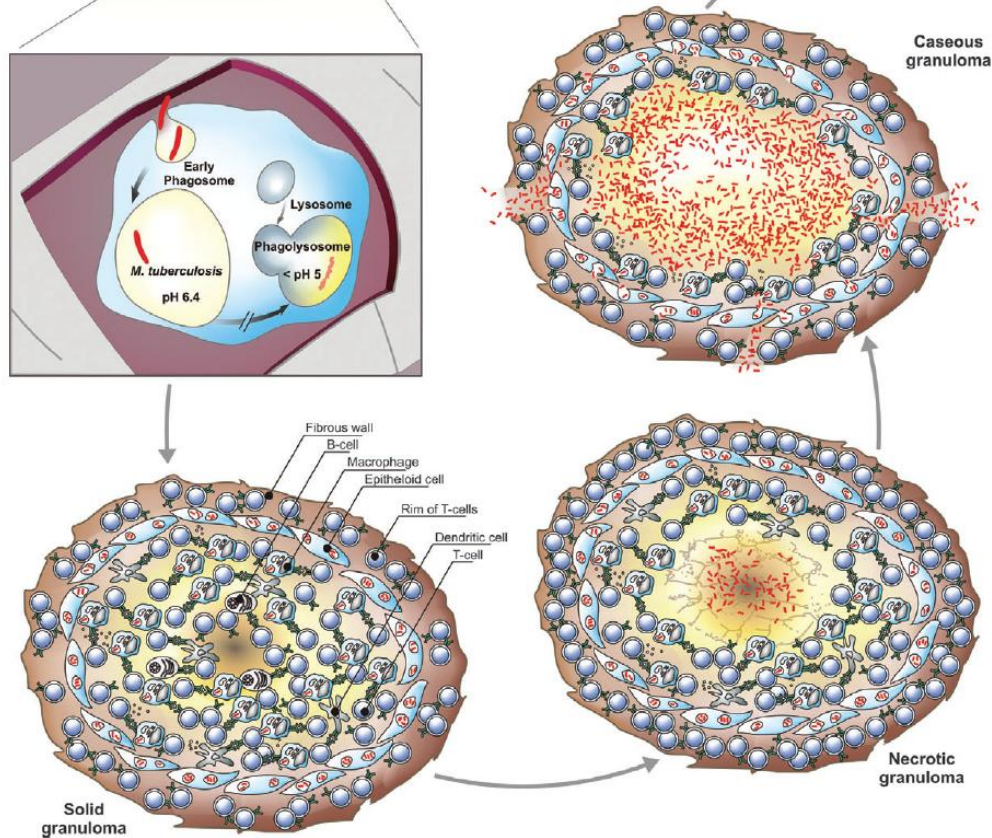
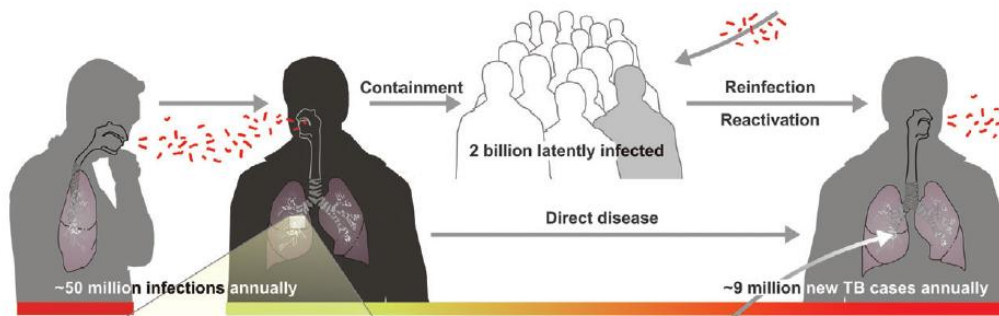


Figure 2 | Positron emission tomography and computed tomography imaging. An ^{18}F -fluorodeoxyglucose (FDG) positron emission tomography (PET) and computed tomography (CT) scan of a patient with tuberculosis with extensive bilateral disease and a complete collapse of the left lung. The right lung also shows extensive disease throughout and illustrates the variability of FDG-PET uptake among lesions within even a single infected patient. The yellow star illustrates one lesion that fails to take up FDG that lies immediately adjacent to a string of three lesions that take up label avidly (red star). These different types of lesion respond to chemotherapy with different kinetics, indicating that they represent distinct bacterial subpopulations in different microenvironments.

Fig. 1. Transmission and pathology of TB. Transmission of TB between individuals occurs via aerosols of infectious bacilli. An estimated 50 million infections per year maintain a pool of ~ 2 billion latently infected individuals. In a few cases, infection directly transforms to active TB. Together with reactivation and reinfection, this gives rise to approximately 9 million new TB cases annually. Upon inhalation of such droplets, the pathogen reaches lung airways and is phagocytosed by alveolar macrophages. The infected host cell induces a localized proinflammatory response that attracts mononuclear cells and T lymphocytes to build up a granuloma, the hallmark tissue reaction of TB. Healthy individuals can control the pathogen at this stage but remain latently infected and thus at risk of reactivation lifelong. Granuloma maturation (solid, necrotic, and caseous) occurs at different velocities and typically culminates in coexistence of all lesion forms during active TB. The caseating granuloma loses solidity because of decay of its center into a structureless accumulation of host cell debris, the caseum. *Mtb* grows to high numbers, is released into airways, and coughed out as contagious aerosol.

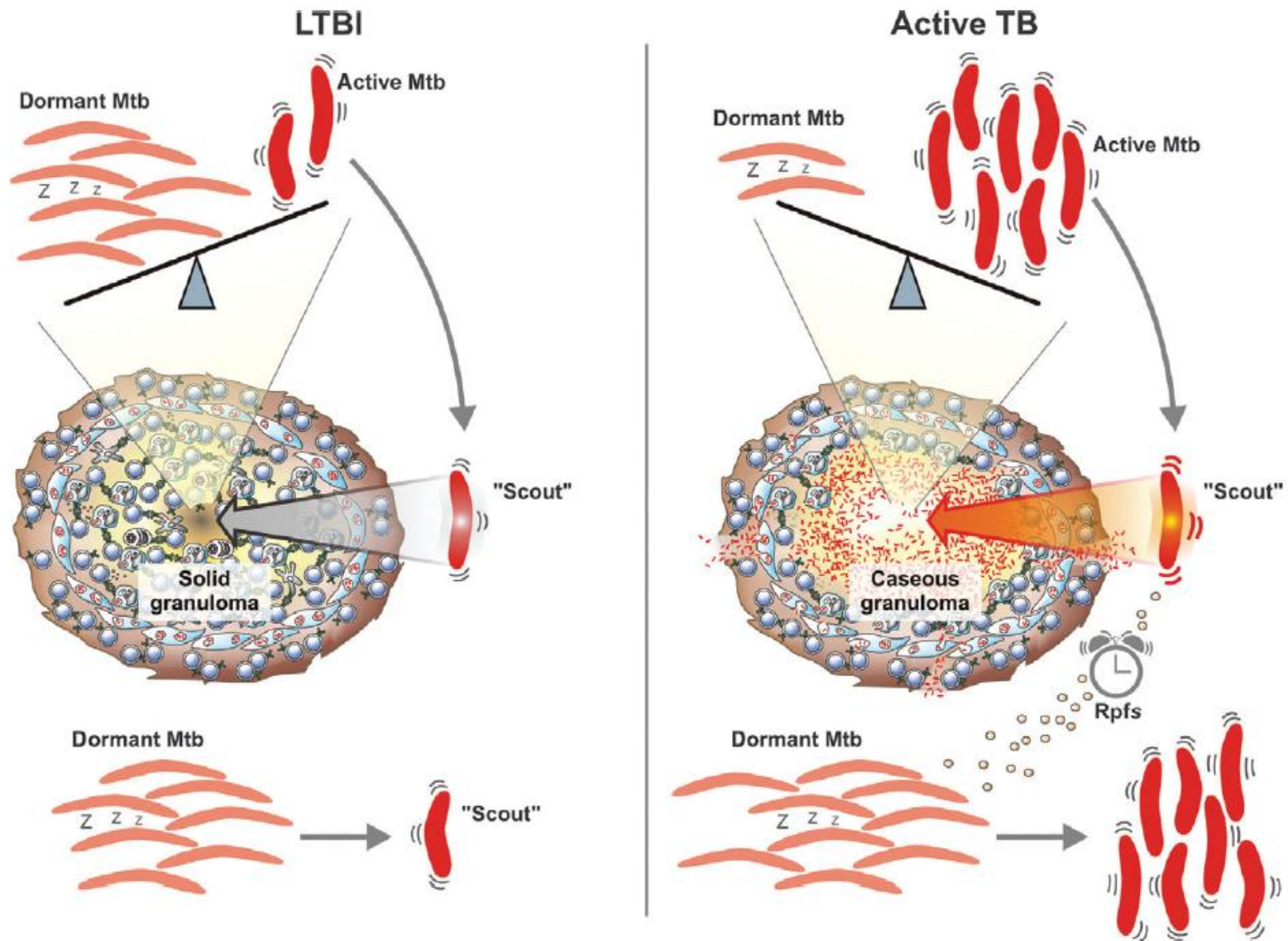


Fig. 3. Dynamic model of latent TB infection (LTBI) and active TB. In this model, LTBI is characterized by predominance of dormant bacilli and only very few active scouts capable of sensing the environment for growth attractiveness, which is low inside the solid granuloma. Some *Mtb* wake-up stochastically to maintain a small pool of scouts (left panel). Once the environment provides more favorable conditions, for example, in a caseating granuloma, scouts resuscitate dormant bacilli to become active probably by secretion of resuscitation-promoting factors (Rpfs) (right panel). A few organisms remain dormant and therefore phenotypically drug resistant, explaining the long treatment time required to cure active TB.

Changing Concepts of “Latent Tuberculosis Infection” in Patients Living with HIV Infection

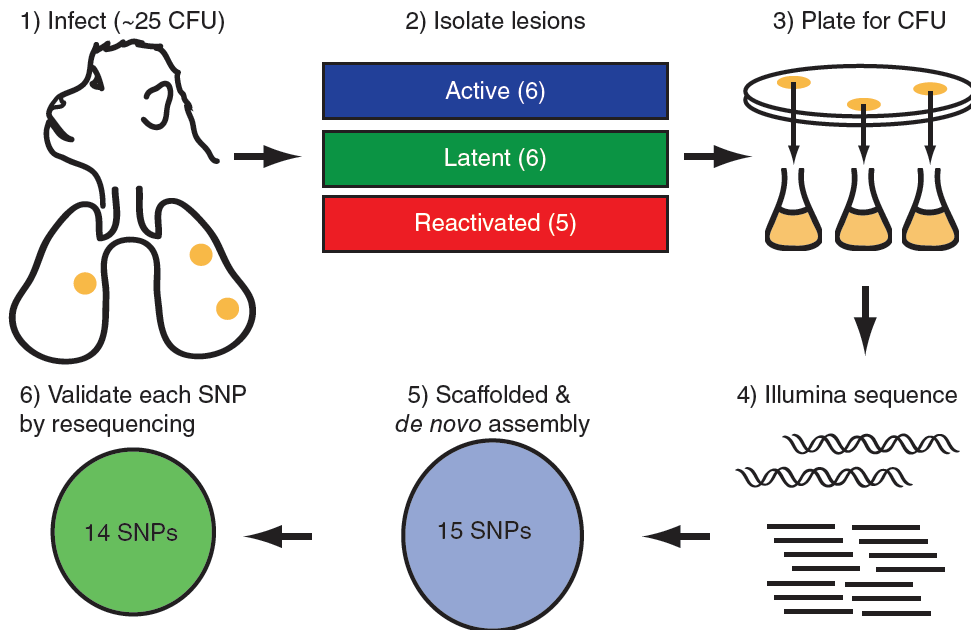
Stephen D. Lawn,^{1,2} Robin Wood,¹ and Robert J. Wilkinson^{3,4,5}

- ✓ **Isoniazid is active against actively replicating organisms and yet reduces TB risk in those with “LTBI”;**
- ✓ **Isoniazid preventive therapy entails 6-12 months of therapy for good efficacy, possibly suggesting the pool of “latent” mycobacteria cycle through phases of metabolic activity and replication over time;**

INH preventive monotherapy (IPT) for LTBI appears to be associated with INH resistance

Use of whole genome sequencing to estimate the mutation rate of *Mycobacterium tuberculosis* during latent infection

Christopher B Ford^{1,11}, Philana Ling Lin^{2,11}, Michael R Chase¹, Rupal R Shah¹, Oleg Iartchouk³, James Galagan⁴⁻⁶, Nilofar Mohaideen⁷, Thomas R Ioerger⁸, James C Sacchettini⁷, Marc Lipsitch^{1,9}, JoAnne L Flynn¹⁰ & Sarah M Fortune¹



- 33 Mtb isolates from nine macaques were sequenced;

Figure 1 Experimental protocol for assessing mutational capacity in different disease states. 1) Cynomolgus macaques were infected with ~25 colony forming units (CFU) of Mtb Erdman using bronchoscopy. 2) Animals were killed in the indicated stages of disease for strain isolation. 3) Eighteen pathologic lesions were plated for bacterial colonies. Thirty-three strains were isolated for WGS. 4) Genomic DNA was isolated from these strains and then analyzed using Illumina sequencing. 5) Reads were assembled using both *de novo* and scaffolded approaches. Fifteen SNPs were predicted by both methodologies. Insertions and deletions were not detected using either methodology. 6) Sanger sequencing confirmed 14 of the 15 putative SNPs identified by both scaffolded and *de novo* analysis.

Use of whole genome sequencing to estimate the mutation rate of *Mycobacterium tuberculosis* during latent infection

Christopher B Ford^{1,11}, Philana Ling Lin^{2,11}, Michael R Chase¹, Rupal R Shah¹, Oleg Iartchouk³, James Galagan⁴⁻⁶, Nilofar Mohaideen⁷, Thomas R Ioerger⁸, James C Sacchettini⁷, Marc Lipsitch^{1,9}, JoAnne L Flynn¹⁰ & Sarah M Fortune¹

Table 1 The predicted mutation rate for biologically relevant generation times

Gen. time (h) (<i>g</i>)	Growth condition	$\mu(g)$ active (95% CI) ^a	$\mu(g)$ latent (95% CI) ^a	$\mu(g)$ reactivated (95% CI) ^a
20	Rich media	2.01×10^{-10} (8.09×10^{-11} to 4.15×10^{-10})	2.71×10^{-10} (5.57×10^{-11} to 7.89×10^{-10})	3.03×10^{-10} (1.22×10^{-10} to 6.24×10^{-10})
45	Macrophage	4.77×10^{-10} (1.30×10^{-10} to 1.22×10^{-9})	5.99×10^{-10} (1.23×10^{-10} to 1.75×10^{-9})	6.71×10^{-10} (2.70×10^{-10} to 1.38×10^{-9})
135	Mouse infection at 10 weeks	1.43×10^{-9} (3.90×10^{-10} to 3.66×10^{-9})	1.80×10^{-9} (3.70×10^{-10} to 5.25×10^{-9})	2.01×10^{-9} (8.09×10^{-10} to 4.15×10^{-9})

The generation time (*g*) was varied from 18–240 h, *t* represents total time of infection in hours and *N* is equal to the number of bases sequenced. The values shown represent the predicted μ and 95% confidence intervals of a bacterial population in animals with active, latent or reactivated disease estimated for the indicated, biologically relevant generation times^{11,12,30}. Gen., generation.

^aMutation rates were estimated using the equation shown: $\mu = m/[N*(t/g)]$, over *g* = 18–240 h.

“We found a similar mutation rate during latency as during active disease or in logarithmically growing culture over the same period of time”

Estimating the mutation rate of *Mycobacterium tuberculosis* during infection

David R Sherman & Sebastien Gagneux

- ✓ **Rischio di comparsa di resistenza all'INH nei casi di profilassi;**
- ✓ **La profilassi quasi mai “sterilizza” dall'infezione. Rischio di riattivazione di un ceppo resistente;**

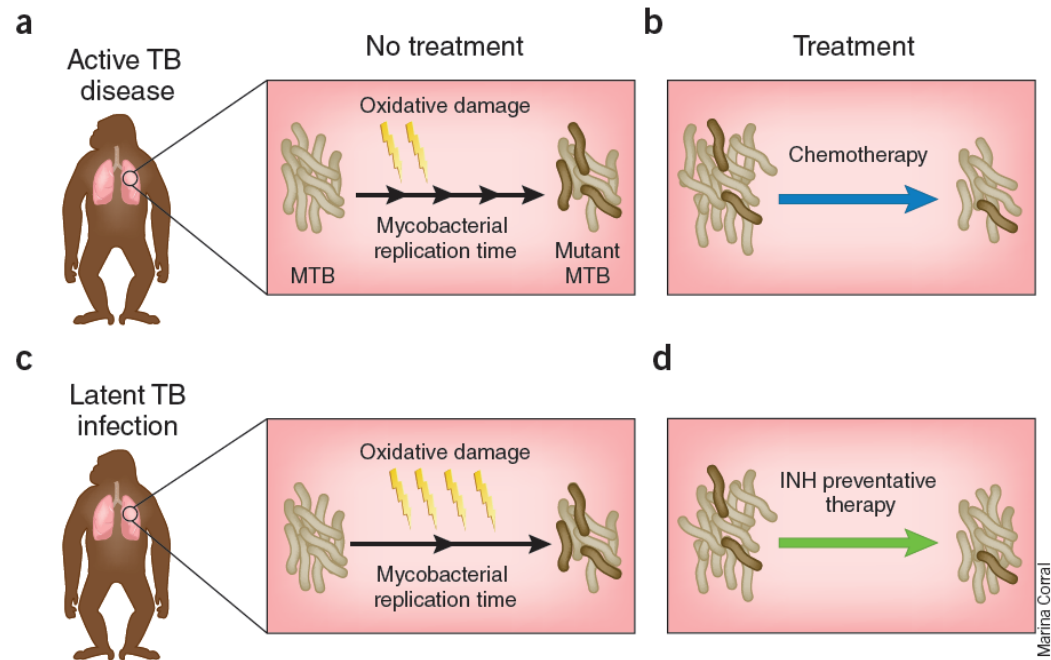


Figure 1 Comparison of *M. tuberculosis* replication and mutation rates within a macaque host with active TB disease or latent infection. (a) During active TB, mycobacteria replicate within the host and increase in numbers. Mutations can arise as a result of either replicative error or oxidative damage. (b) Active TB disease is treated with a combination of drugs (chemotherapy), which greatly reduces mycobacterial numbers. (c) During latent TB infection, although there is a reduced level of replication, Ford *et al.*² find that a similar number of mutations arise within the host mycobacterial population. These mutations may be attributable to increased levels of oxidative stress. (d) Latent TB infection is treated with a single drug, isoniazid (INH). Ford *et al.*² show that during latency, Mtb retains mutational capacity, which may contribute to the epidemiological link between INH preventative therapy and the subsequent development of INH-resistant TB.

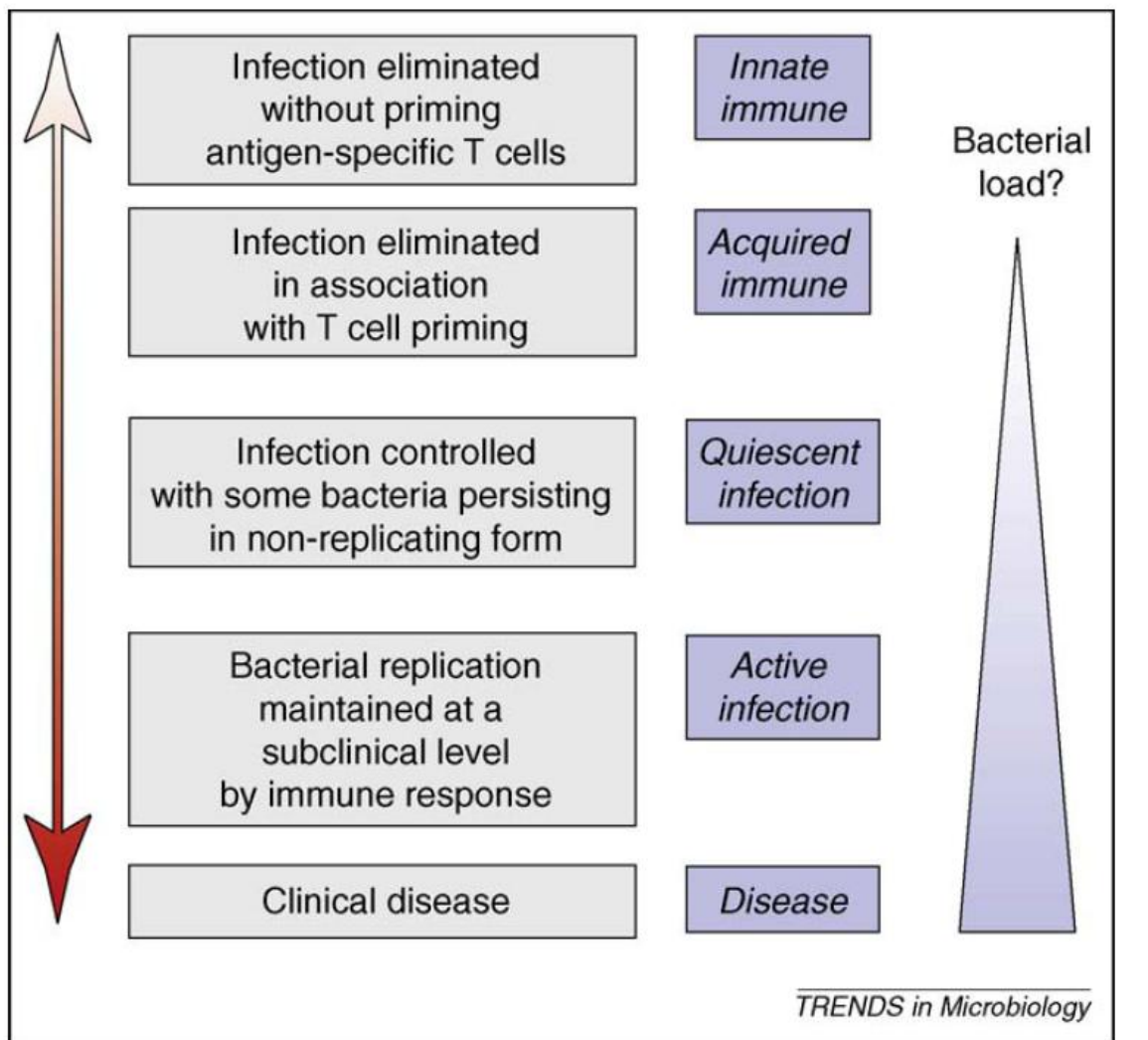
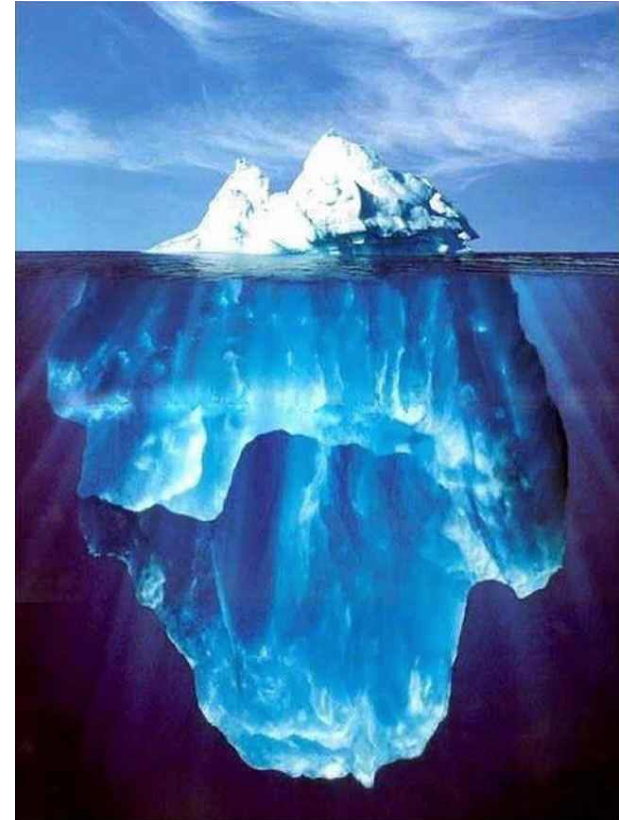


Figure 2. A spectrum of responses to tuberculosis infection. Infection with *M. tuberculosis* is usually viewed in terms of a binary outcome: as active disease or latent infection. We propose that a model that includes a spectrum of responses provides a better representation of the biology of infection and might assist in formulation of appropriate research questions.



Changing Concepts of “Latent Tuberculosis Infection” in Patients Living with HIV Infection

Stephen D. Lawn,^{1,2} Robin Wood,¹ and Robert J. Wilkinson^{3,4,5}

- ✓ **Risk of developing active TB in patients with “LTBI” varies considerably over time, suggesting that latent infection is a heterogeneous state;**
- ✓ **A significant proportion of patients with a microbiologically proven pulmonary TB identified by prevalence surveys have no symptoms;**
- ✓ **Mycobacterial lesions within tissues from the same individual may represent a wide spectrum, ranging from sterility to multi-bacillary disease;**

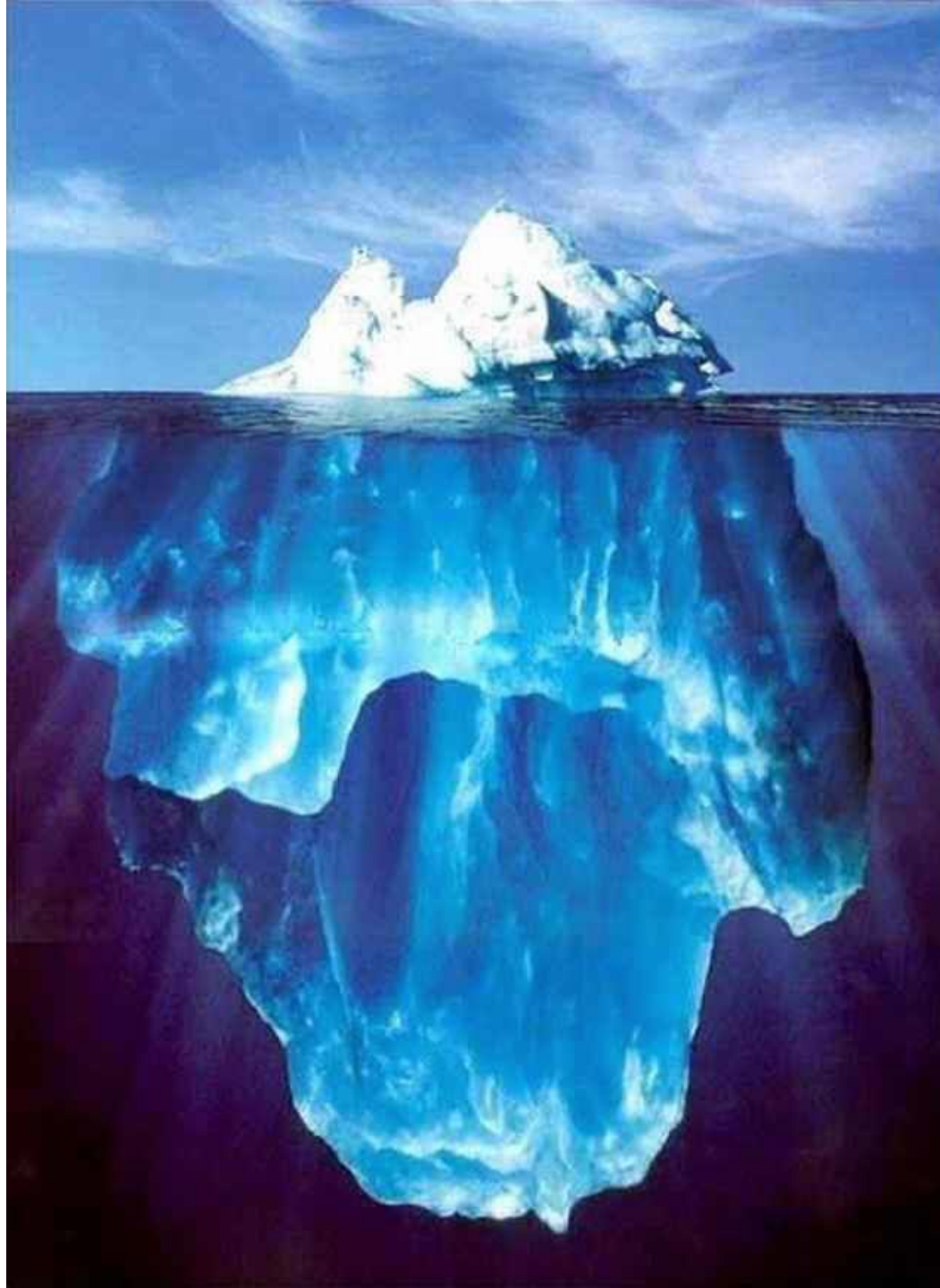
DIRETTA

Microbiologica

INDIRETTA

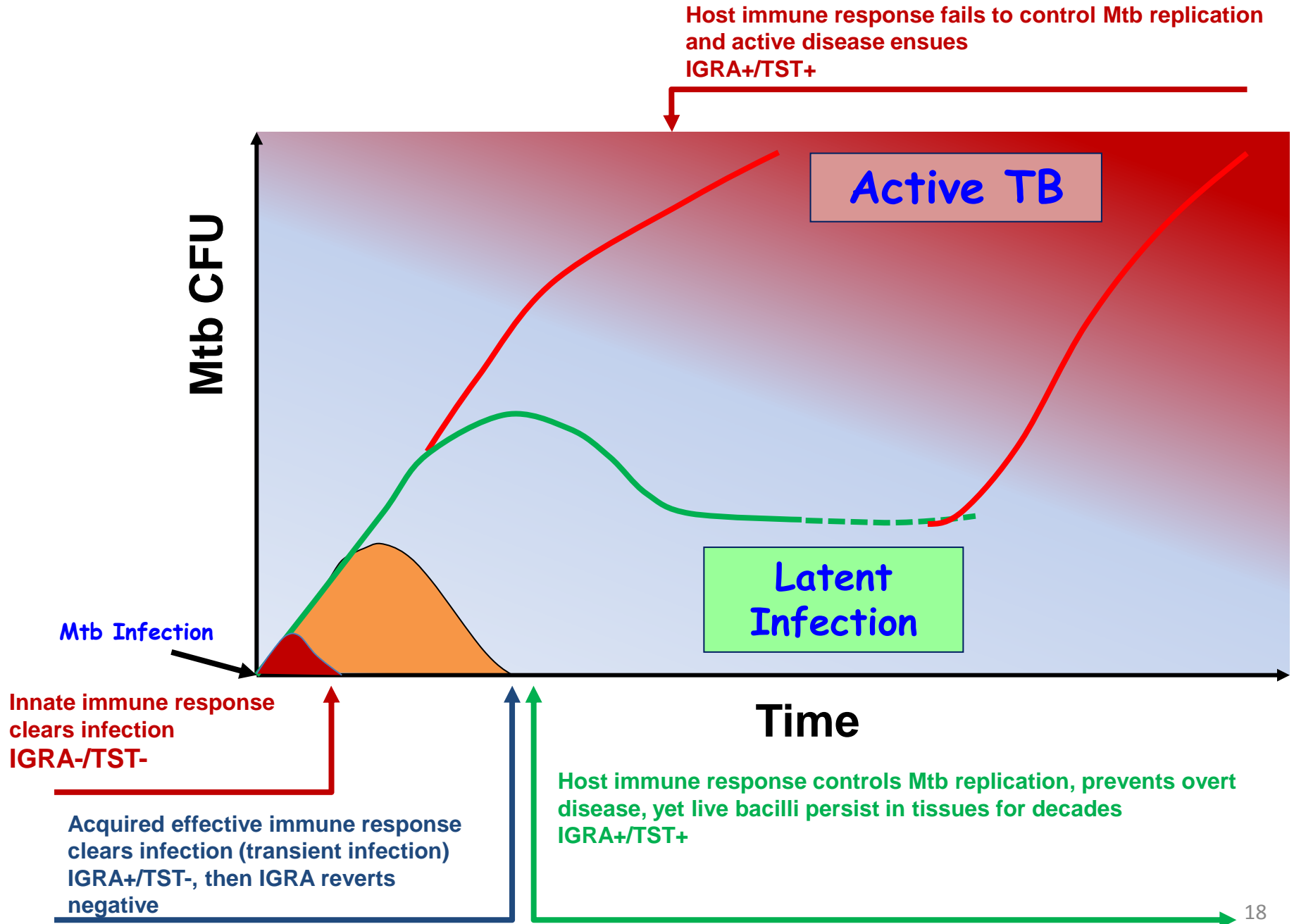
Immunologica

- TST (PPD)
- IGRAs (RD1)
 - QFT
 - TBspot



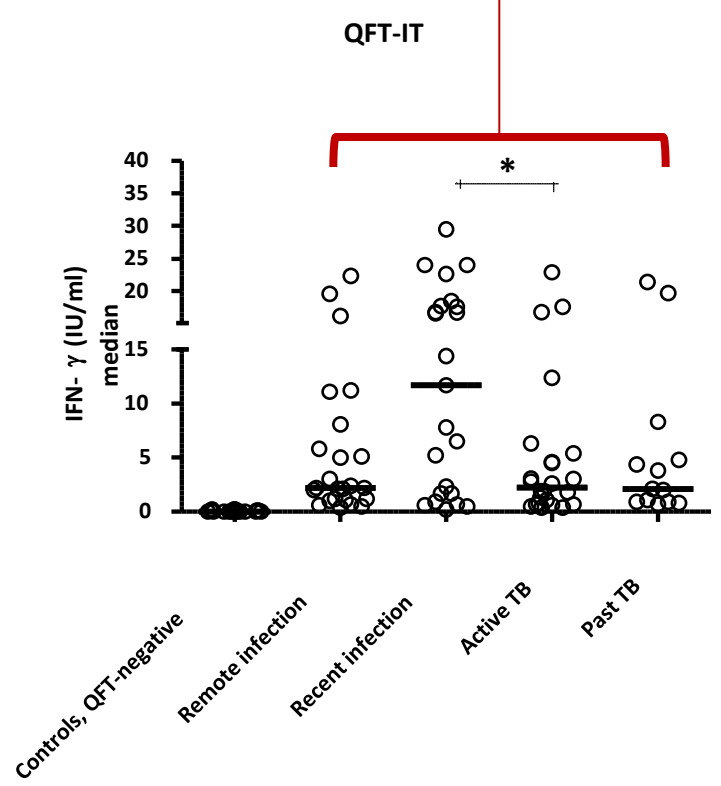
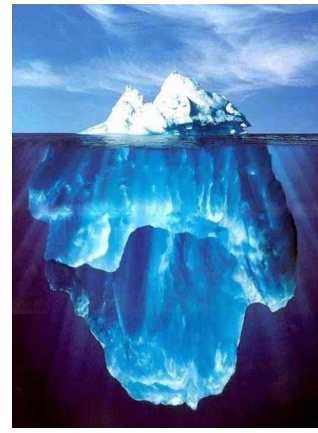
**TB
ATTIVA**

**TB
LATENTE**



Methylated HBHA Produced in *M. smegmatis* Discriminates between Active and Non-Active Tuberculosis Disease among RD1-Responders

Giovanni Delogu¹, Teresa Chiacchio², Valentina Vanini², Ornella Butera², Gilda Cuzzi², Alessandra Bua³, Paola Molicotti³, Stefania Zanetti³, Francesco Nicola Lauria⁴, Susanna Grisetti⁵, Nicola Magnavita⁶, Giovanni Fadda¹, Enrico Girardi⁷, Delia Goletti^{2*}



Risultati del QFT-IT in UI di IFN-γ per ml

UI/ml di IFN-γ Ag Mtb	Mitogeno	Risultato
<0,35	positivo	NEGATIVO
<0,35	negativo	INDETERMINATO
>0,35	Positivo o negativo	POSITIVO

Spectrum of TB infection

- ✓ **Quale è il significato clinico dell'eterogeneità dell'infezione tubercolare?**
- ✓ **E' possibile individuare parametri immunologici che possano descrivere questa complessità?**
- ✓ **E' possibile individuare i soggetti con aumentato rischio di sviluppare malattia attiva?**

Gruppi con aumentato rischio per lo sviluppo di TB

- ✓ **Bambini (< 5 anni);**
- ✓ **Soggetti immunocompromessi (HIV, etc.);**
- ✓ **Soggetti da sottoporre a farmaci immunodepressivi
(cortisone, chemioterapie, farmaci biologici, etc.);**

Infectious complications of tumor necrosis factor blockade

Robert S. Wallis

Pfizer, New London, Connecticut, USA

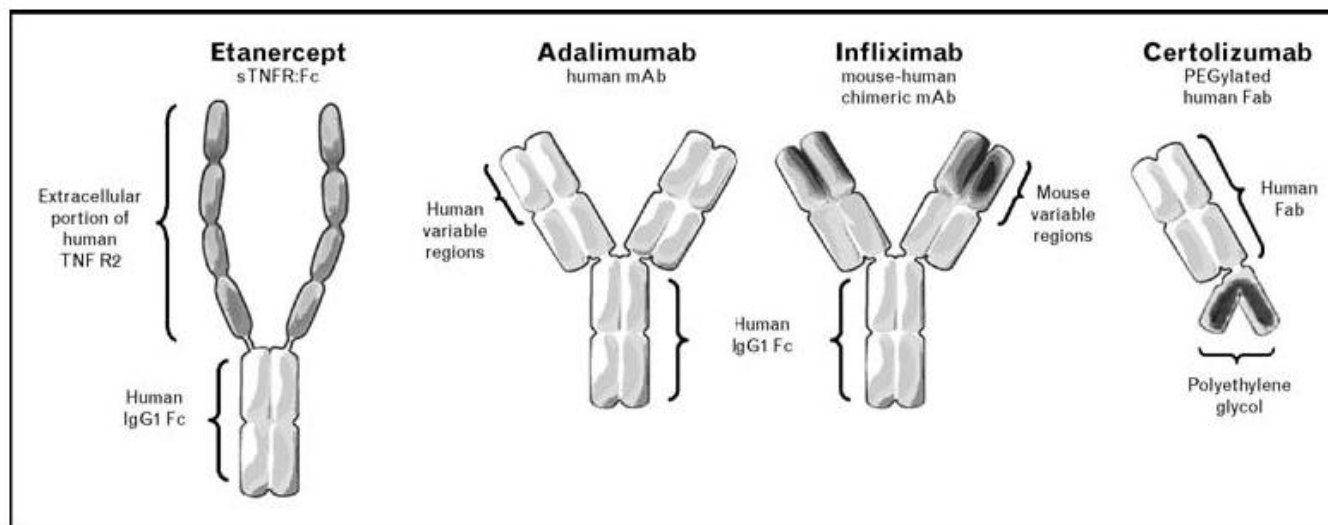
Correspondence to Dr Robert S. Wallis, MD, FIDSA, Pfizer, 50 Pequot Avenue, B3149, New London, Connecticut, 06320 USA
Tel: +1 860 732 2573; e-mail: rswallis@gmail.com

Current Opinion in Infectious Diseases 2009, 22:403–409

Purpose of review

Our understanding of the infection risks posed by tumor necrosis factor (TNF) antagonists has continued to evolve in the 10 years since these drugs were first introduced. This review summarizes recent data regarding infection risk, examines potential structure–function relationships that may account for the differences, and discusses their implications with regard to tuberculosis prevention and management.

Figure 1 Structures of the tumor necrosis factor antagonists



Adapted with permission from [1**]. mAb, monoclonal antibody; TNFR, tumor necrosis factor receptor.

Anti-TNF immunotherapy reduces CD8⁺ T cell-mediated antimicrobial activity against *Mycobacterium tuberculosis* in humans

Heiko Bruns,¹ Christoph Meinken,² Philipp Schauenberg,³ Georg Härter,⁴ Peter Kern,⁴ Robert L. Modlin,⁵ Christian Antoni,³ and Steffen Stenger¹

¹Institute for Medical Microbiology and Hygiene, University Hospital of Ulm, Ulm, Germany. ²Institute for Clinical Microbiology, Immunology and Hygiene, and ³Department for Internal Medicine 3, University Erlangen-Nürnberg, Erlangen, Germany. ⁴Section of Infectious Diseases and Clinical Immunology, University Hospital of Ulm, Ulm, Germany. ⁵Department of Medicine, Division of Dermatology, UCLA David Geffen School of Medicine, Los Angeles, California, USA.

Anti-TNF immunotherapy and tuberculosis reactivation: another mechanism revealed

Elizabeth A. Miller^{1,2} and Joel D. Ernst^{1,3,4}

¹Division of Infectious Diseases, Department of Medicine, ²Cancer Institute, ³Department of Pathology, and ⁴Department of Microbiology, New York University School of Medicine, New York, New York, USA.

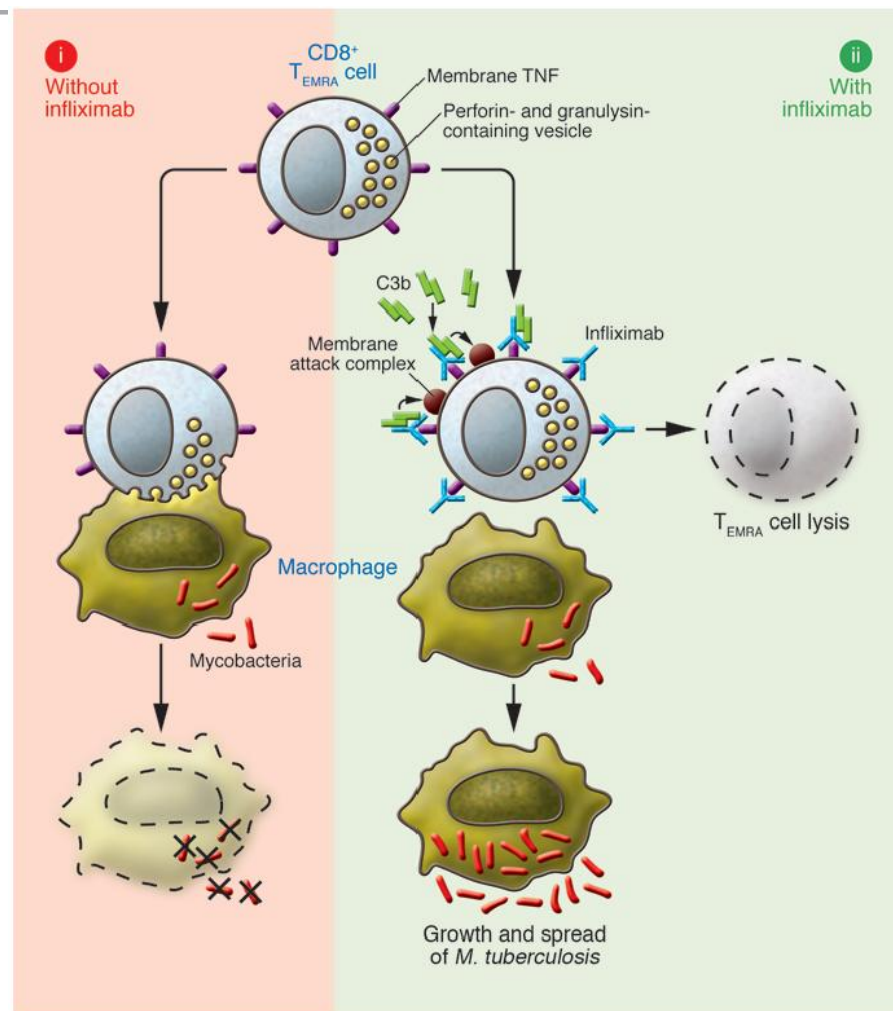
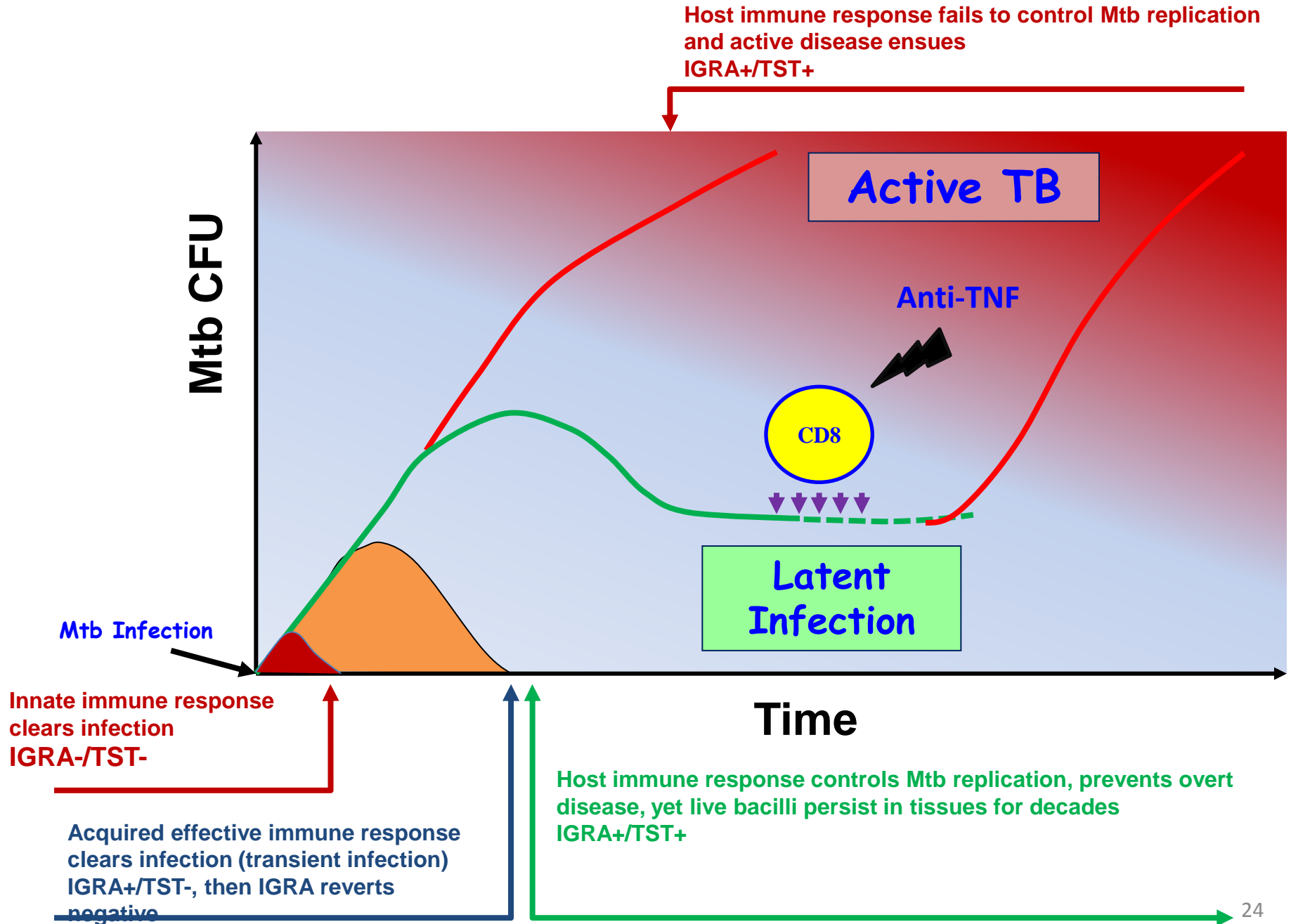


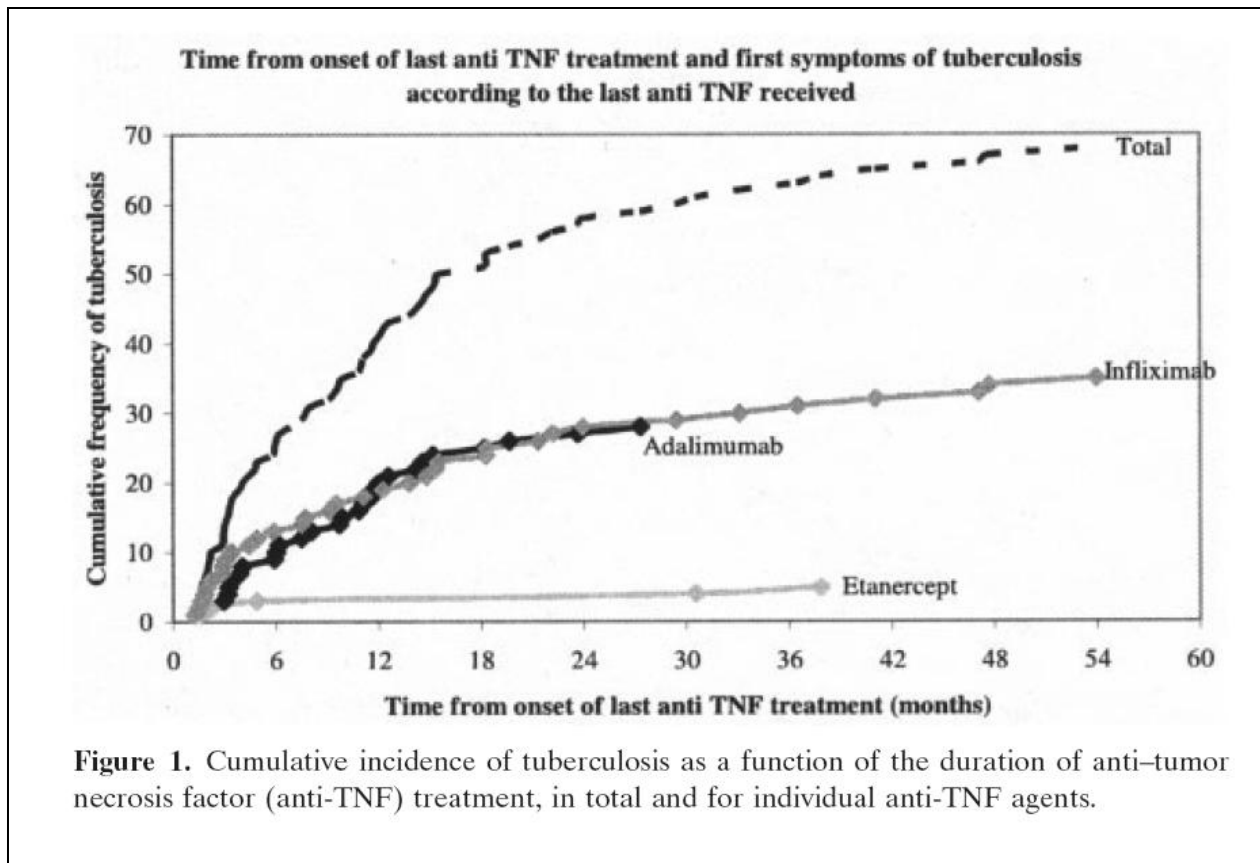
Figure 1
Effect of TNF neutralization with infliximab on the antimycobacterial action of CD8⁺ T_{EMRA} cells. In the absence of the TNF-neutralizing drug infliximab (i), cytotoxic T_{EMRA} cells are present and release their granules containing perforin and granulysin, resulting in the death of *M. tuberculosis*-infected macrophages and intracellular and extracellular mycobacteria. In this issue of the *JCI*, Bruns et al. (3) report that in the presence of infliximab (ii), membrane TNF on T_{EMRA} cells is bound by the antibody, and CDC ensues. The depletion of T_{EMRA} cells results in suboptimal control of mycobacterial growth, leading to the potential spread of *M. tuberculosis* infection.

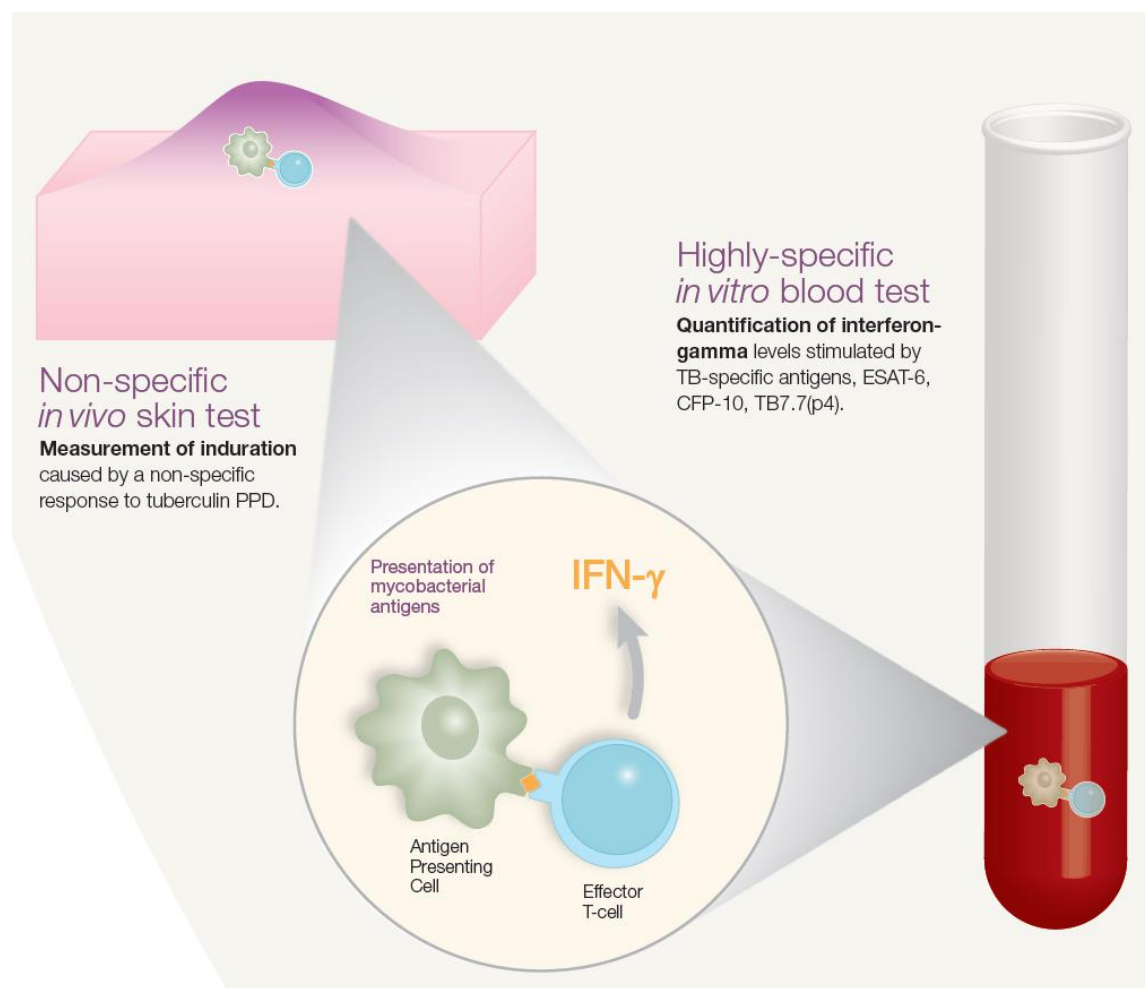


Risk of Tuberculosis Is Higher With Anti-Tumor Necrosis Factor Monoclonal Antibody Therapy Than With Soluble Tumor Necrosis Factor Receptor Therapy

The Three-Year Prospective French Research Axed on Tolerance of Biotherapies Registry

F. Tubach,¹ D. Salmon,² P. Ravaud,¹ Y. Allanore,³ P. Goupille,⁴ M. Bréban,⁵ B. Pallot-Prades,⁶ S. Pouplin,⁷ A. Sacchi,⁸ R. M. Chichemanian,⁹ S. Bretagne,¹⁰ D. Emilie,¹¹ M. Lemann,¹² O. Lortholary,¹³ and X. Mariette,¹⁴ for the Research Axed on Tolerance of Biotherapies Group





Un test IGRA positivo indica la presenza di linfociti T effettori e quindi di antigeni RD1 disponibili *in vivo*

Infezione con *M. tuberculosis*

Clinical applicability of Quantiferon-TB-Gold testing in psoriasis patients during long-term anti-TNF-alpha treatment: a prospective, observational study

S. Garcovich,^{†,*} A. Ruggeri,[‡] M. D'Agostino,[†] F. Ardito,[‡] C. De Simone,[†] G. Delogu,[‡] G. Fadda[‡]

[†]Department of Internal Medicine and Specialist Sciences, Institute of Dermatology, A. Gemelli University Hospital, Catholic University of Sacred Heart, Rome, Italy

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*Correspondence: S. Garcovich. E-mail: simgarko@yahoo.it

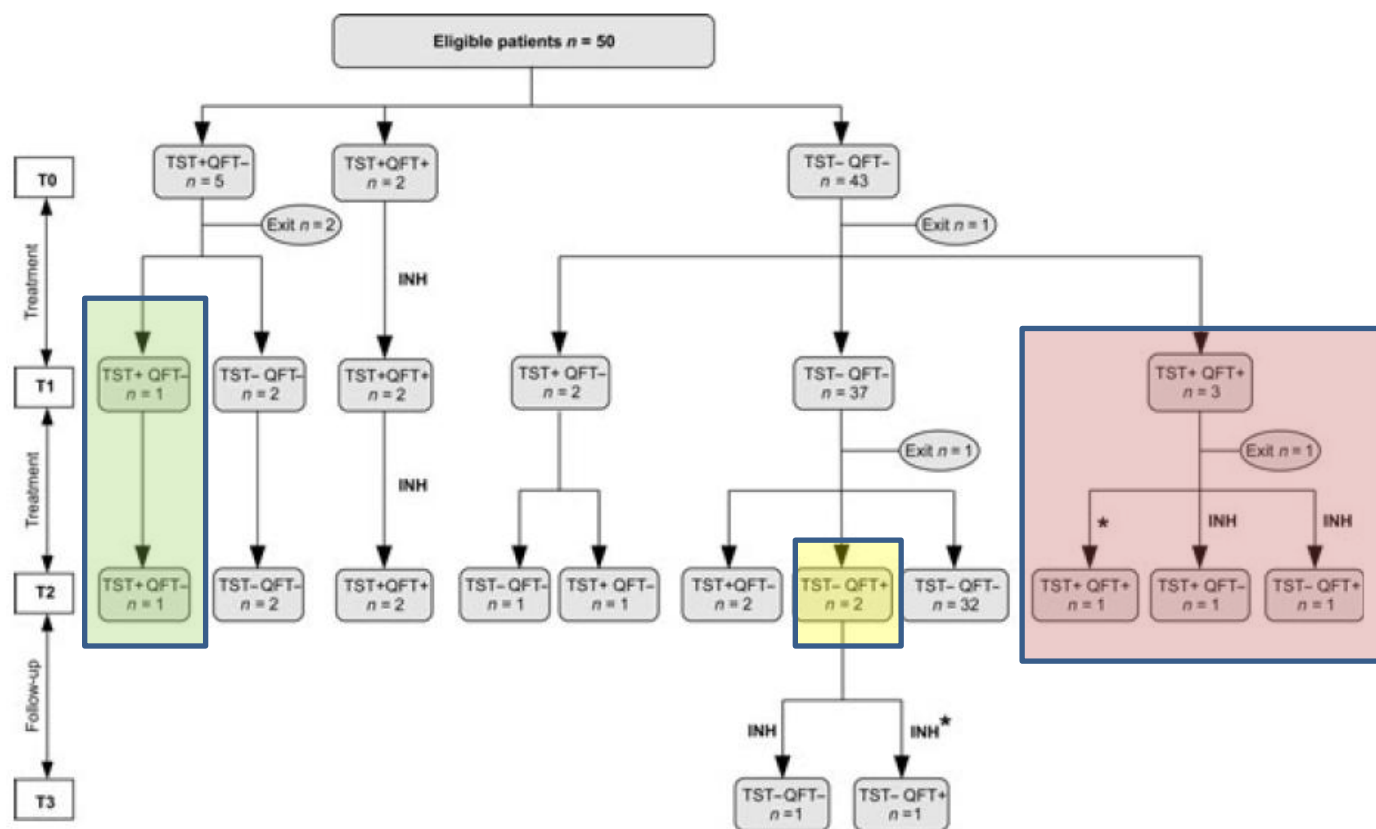


Figure 1 Study flow-chart distribution of tuberculin skin test (TST) and Quantiferon-TB-Gold (QFT) results of 50 patients at baseline (screening), after 6 months (T1), after 12 months (T2) of continuous anti-TNF-alpha treatment and after 6 months of follow-up (T3). INH = isoniazid treatment; * = anti-TNF-alpha treatment stopped due to contraindications/side-effects of isoniazid chemoprophylaxis.



Damage + PAMPs

mechanical
toxic (Phenol)

contained in
PPD ?



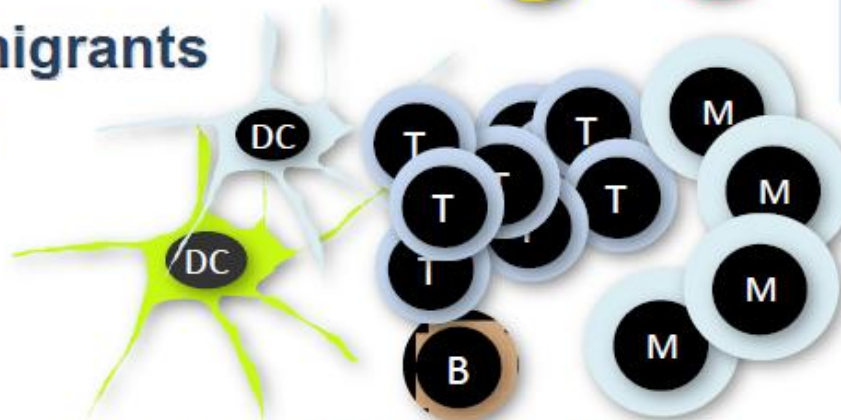
**Sentinel cells
(in tissue)**



**Early immigrants
(< 6h)**



**Later immigrants
(12 - 48h)**



Mediator release

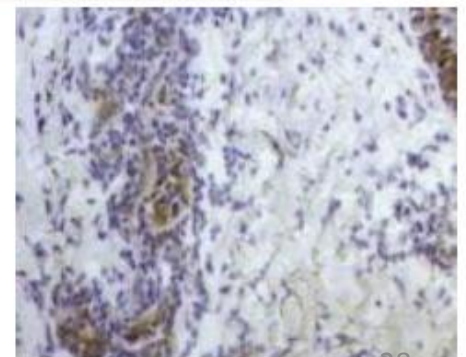
- TNF α (Mast cells)
- Eicosanoids
- Histamin, a.o.

Cytokines/Enzymes

- Interleukins, IFNs
- Proteases
- Chemokines

Antigen Processing & Presentation

Adaptive Innate



RESEARCH ARTICLE

Deciphering the proteome of the in vivo diagnostic reagent “purified protein derivative” from *Mycobacterium tuberculosis*

Yun Sang Cho^{1*,**}, Karen M. Dobos^{1*}, Jessica Prenni², Hongliang Yang¹, Ann Hess³, Ida Rosenkrands⁴, Peter Andersen⁴, Sung Weon Ryou⁵, Gill-Han Bai⁵, Michael J. Brennan^{6**}, Angelo Izzo¹, Helle Bielefeldt-Ohmann^{1**} and John T. Belisle¹

✓ Four heat shock proteins dominate the composition of PPD (solubility and immunological activity);

✓ Variance among different PPD preparations in the relative amount of certain antigens (for instance EsxB!!!) and in the DTH responses elicited in guinea pigs;

Interferon- γ Release Assay Versus Tuberculin Skin Test
 Prior to Treatment With Golimumab,
 a Human Anti-Tumor Necrosis Factor Antibody,
 in Patients With Rheumatoid Arthritis, Psoriatic Arthritis, or
 Ankylosing Spondylitis

Elizabeth C. Hsia,¹ Neil Schluger,² John J. Cush,³ Richard E. Chaisson,⁴ Eric L. Matteson,⁵
 Stephen Xu,⁶ Anna Beutler,⁶ Mittie K. Doyle,¹ Benjamin Hsu,⁶ and Mahboob U. Rahman¹

Table 3. Results of the tuberculin skin test (TST) and interferon- γ release assay (IGRA) in 2,282 patients with both tests performed at screening

Screening test result	No. (%) of patients
IGRA/TST	
IGRA positive	
TST positive	59
TST negative	101
IGRA negative	
TST positive	150
TST negative	1,931
IGRA indeterminate	
TST positive	6
TST negative	35
Overall	
Positive by TST only	215 (9.4)
Positive by IGRA only	160 (7.0)
Positive by both TST and IGRA	59 (2.6)
Positive by either TST or IGRA	316 (13.8)

Table 4. Results of the tuberculin skin test (TST) and interferon- γ release assay (IGRA) in patients with and those without prior bacillus Calmette-Guérin (BCG) vaccination*

Screening test result	Prior BCG vaccination (n = 781)	No prior BCG vaccination (n = 1,248)
IGRA/TST		
IGRA positive		
TST positive	28	24
TST negative	43	48
IGRA negative		
TST positive	91	32
TST negative	610	1,120
IGRA indeterminate		
TST positive	0	6
TST negative	9	18
Overall		
Positive by TST only	119 (15.2)	62 (5.0)
Positive by IGRA only	71 (9.1)	72 (5.8)
Positive by both TST and IGRA	28 (3.6)	24 (1.9)
Positive by either TST or IGRA	162 (20.7)	110 (8.8)

* Values are the number (%) of patients.

Discordance between TST and IGRAs results cannot simply be explained by differences in specificity. These assays appear to reflect different aspects of immune sensitization which are incompletely understood;

The prognosis of latent tuberculosis: can disease be predicted?

Peter Andersen¹, T. Mark Doherty¹, Madhukar Pai² and Karin Weldingh¹

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²McGill University, Department of Epidemiology, Biostatistics & Occupational Health, Montreal H3A 1A2, Quebec, Canada

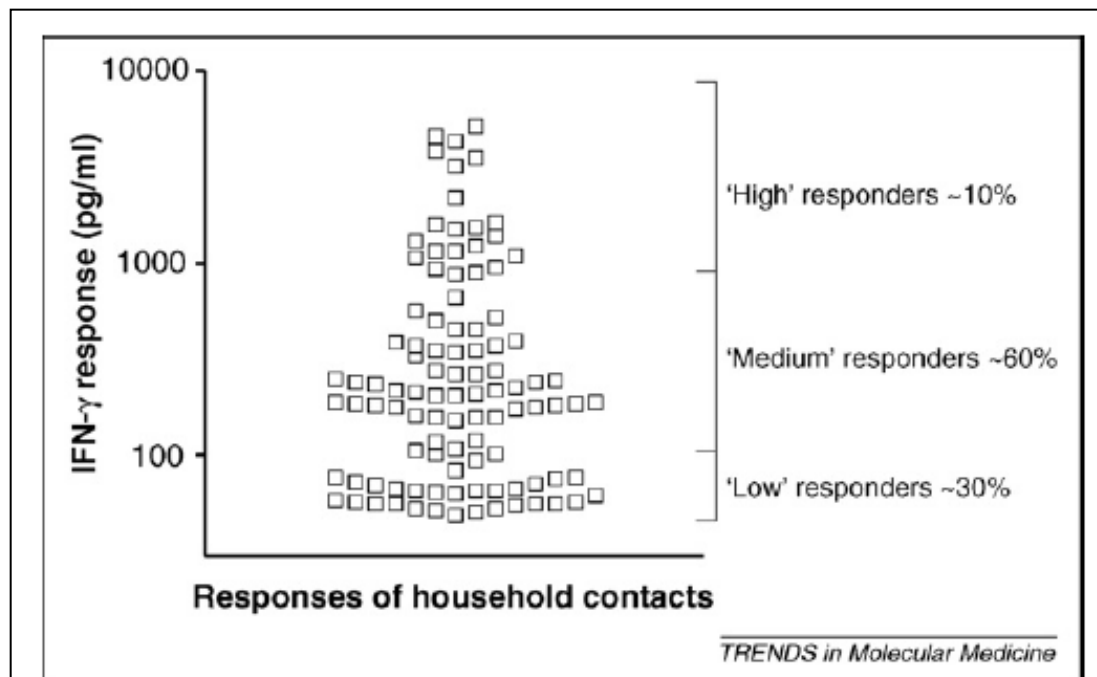
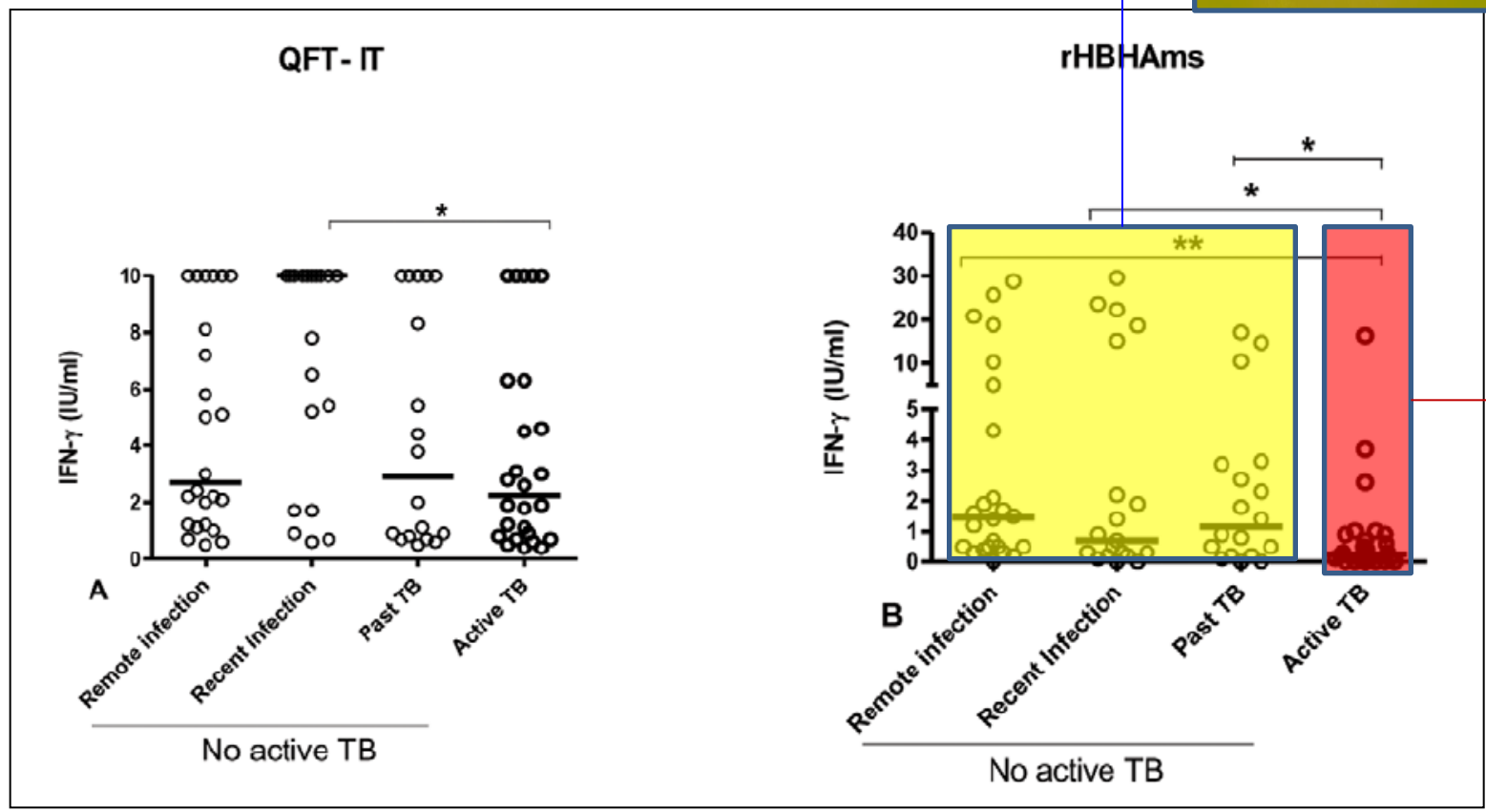
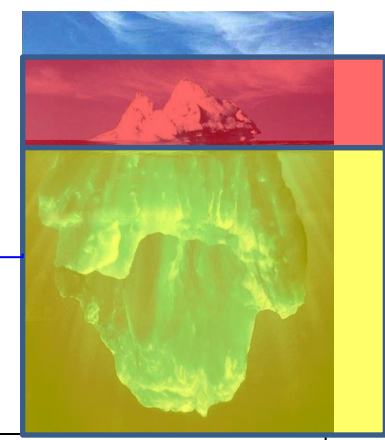
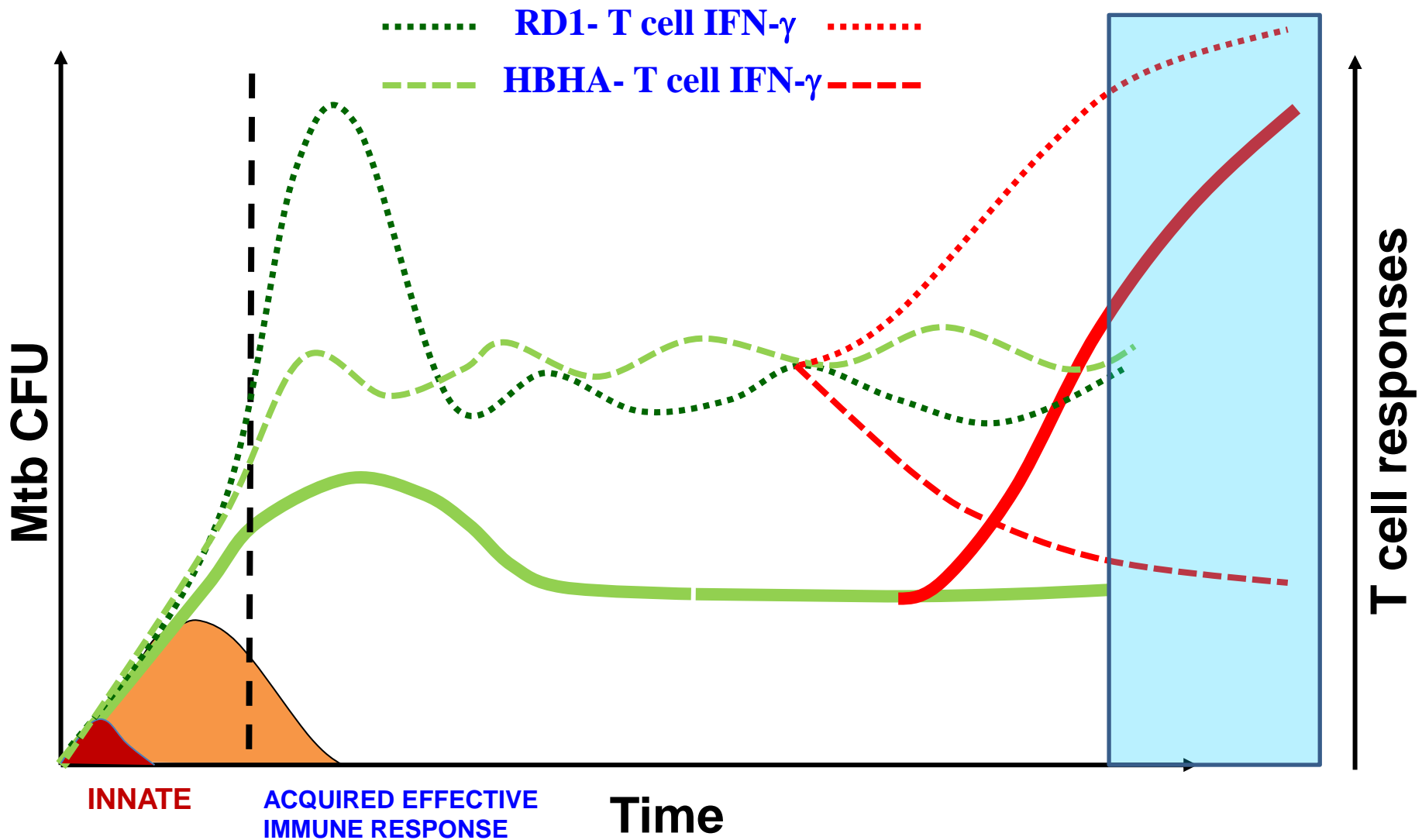


Figure 2. Distribution of ESAT-6 responses among TB contacts in high endemic settings (e.g. Ethiopia). This figure shows that the immune responses (assessed here as IFN- γ production) to ESAT-6 after exposure to infectious TB patients are not normally distributed. The subjects are grouped into low (<100 pg/ml), medium (100–1000 pg/ml) and high (>1000 pg/ml) responders, based on the distribution of the samples. Data are derived from [52].

Methylated HBHA Produced in *M. smegmatis* Discriminates between Active and Non-Active Tuberculosis Disease among RD1-Responders

Giovanni Delogu¹, Teresa Chiacchio², Valentina Vanini², Ornella Butera², Gilda Cuzzi², Alessandra Bua³, Paola Mollicotti³, Stefania Zanetti³, Francesco Nicola Lauria⁴, Susanna Grisetti⁵, Nicola Magnavita⁶, Giovanni Fadda¹, Enrico Girardi⁷, Delia Goletti^{2*}





STORIA NATURALE DELLA TB

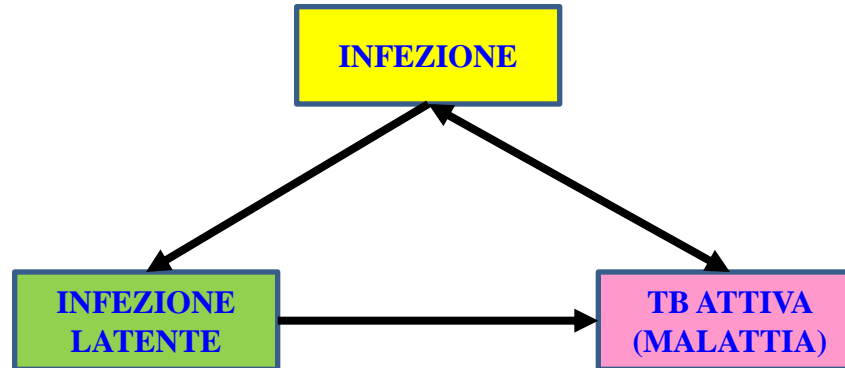
Sommario

- ✓ **Quale test per fare diagnosi di TB latente?**
- ✓ **E' possibile individuare i soggetti con aumentato rischio per lo sviluppo di TB attiva?**
- ✓ **Quale significato dare al valore di IFN-gamma? Cut-off in pazienti trattati con anti-TNF?**
- ✓ **Come e quando iniziare la profilassi?**
- ✓ **Quale è il significato delle conversioni/reversioni?**
- ✓ **Quale è il rischio di (nuova) infezione durante il trattamento con biologici?**
- ✓ **E' importante il monitoraggio dell'infezione (tra i negativi)?**

La diagnosi di TB nei bambini

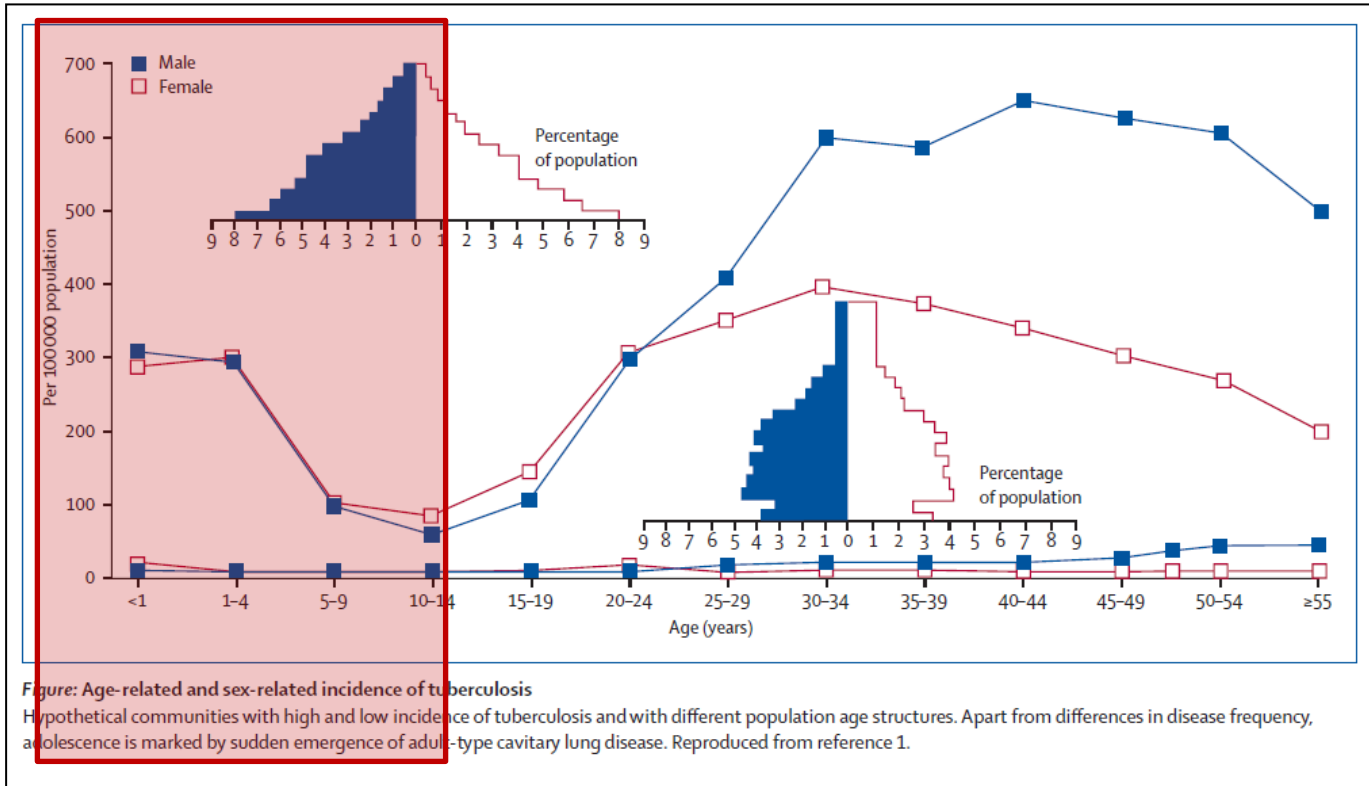
- ✓ I bambini sono particolarmente suscettibili allo sviluppo della TB attiva a seguito di infezione con *M. tuberculosis*;
- ✓ Aumentato rischio di manifestazioni della malattia particolarmente gravi (disseminata, meningea);
- ✓ Importanza di una rapida ricerca dei contatti ed attivazione della profilassi nei bambini infetti;

Diagnosi delle infezioni da *M. tuberculosis*



Il sospetto di TB emerge tenendo conto di numerosi parametri:

- **Clinici;**
- **Epidemiologici;**
- **Categorie a rischio;**



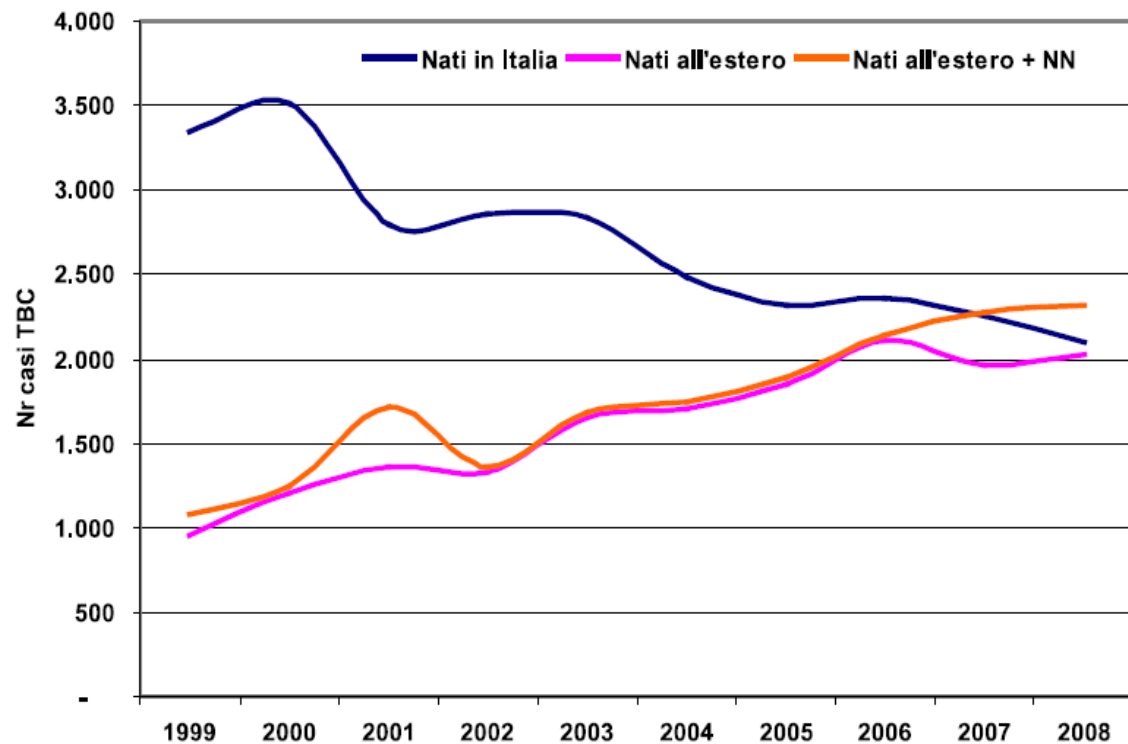
Donald PR, et al (2010) Lancet 375

Cittadini nati all'estero

NUMERO DI CASI DI TBC E INCIDENZA

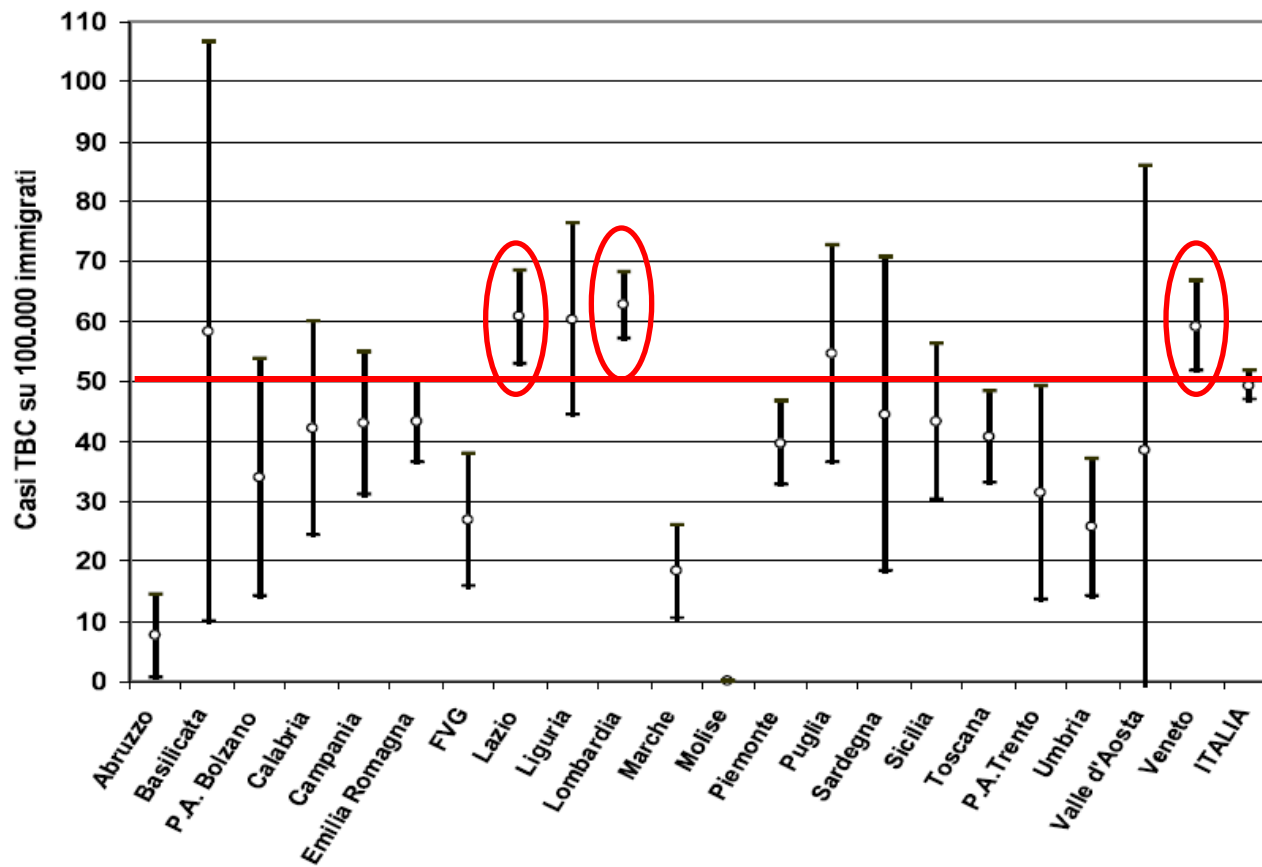
Dal 1999 al 2008, i casi di TBC registrati in "cittadini nati all'estero" hanno rappresentato, nel complesso, il 36,5% del totale dei casi notificati in Italia. Nel periodo esaminato si è verificato un costante aumento di tale proporzione (dal 22% del 1999 al 46% nel 2008). Nel 2008 sono stati notificati 2026 casi di TBC in cittadini stranieri a fronte dei 2102 casi in italiani (in 290 casi non era noto il paese di nascita, il 6,6% dei casi, [Figura 4.10](#)).

Figura 4.10. Casi di TBC per Paese di nascita dal 1999 al 2008



Fonte: Ministero della salute
- Direzione generale della
prevenzione sanitaria, Ufficio
V Malattie Infettive e profilassi
internazionale

Figura 4.12. Stime dei tassi di incidenza della TBC negli immigrati per Regione (IC95%) *



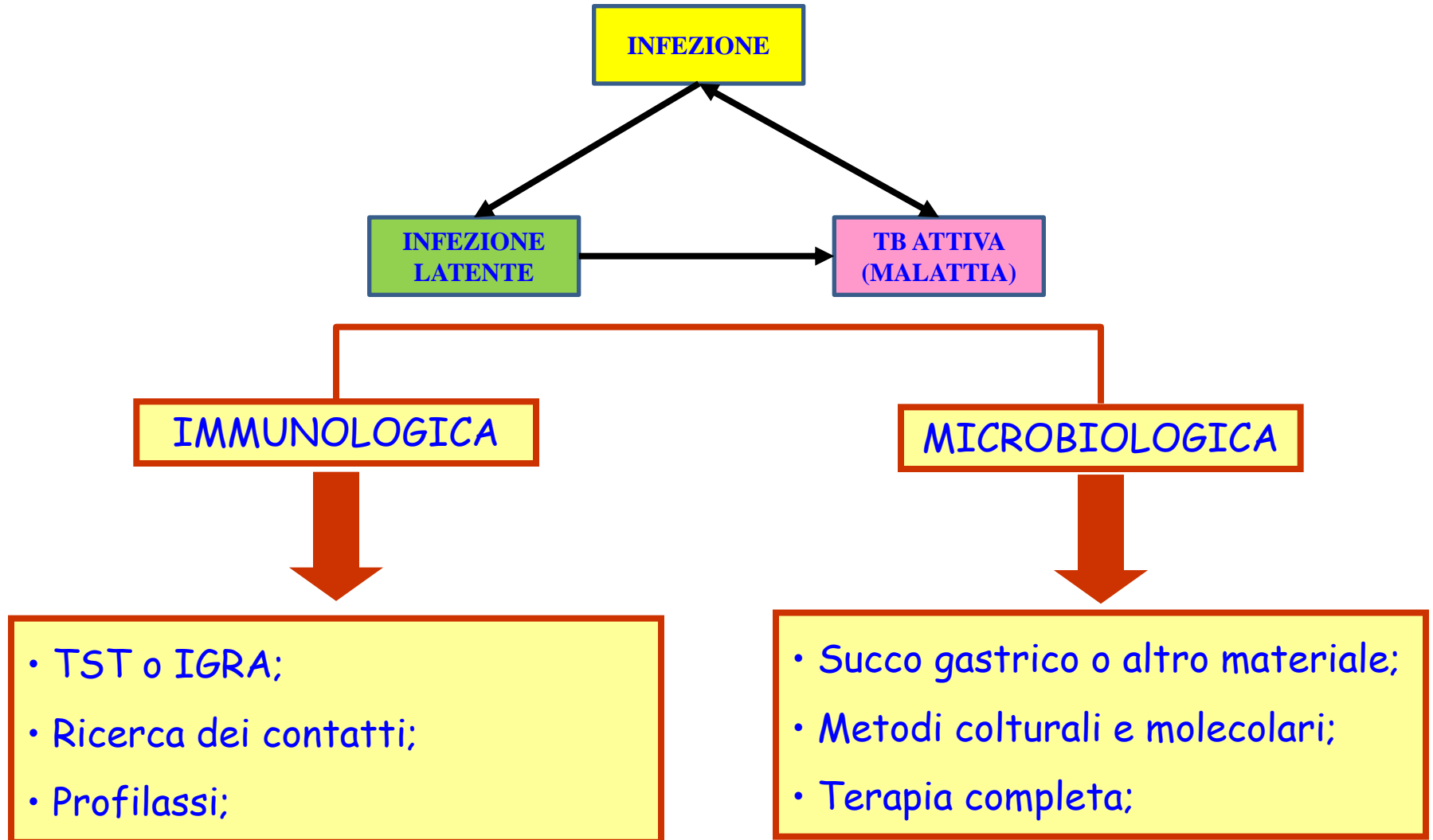
* Denominatore: stima del numero massimo di immigrati presenti sul territorio.

Fonte: Dossier Statistico Immigrazione 2008

La TB nei bambini

- ✓ **Il cambiamento del quadro epidemiologico in Italia richiede l'adozione di nuovi strumenti di controllo e prevenzione;**
- ✓ **Aumentata attenzione in particolari popolazioni;**
- ✓ **Rischio di microepidemie;**
- ✓ **Gestione del problema da un punto di vista non solo sanitario;**

Diagnosi delle infezioni da *M. tuberculosis*





A Twenty-year Retrospective Study of Pediatric Tuberculosis in Two Tertiary Hospitals in Rome

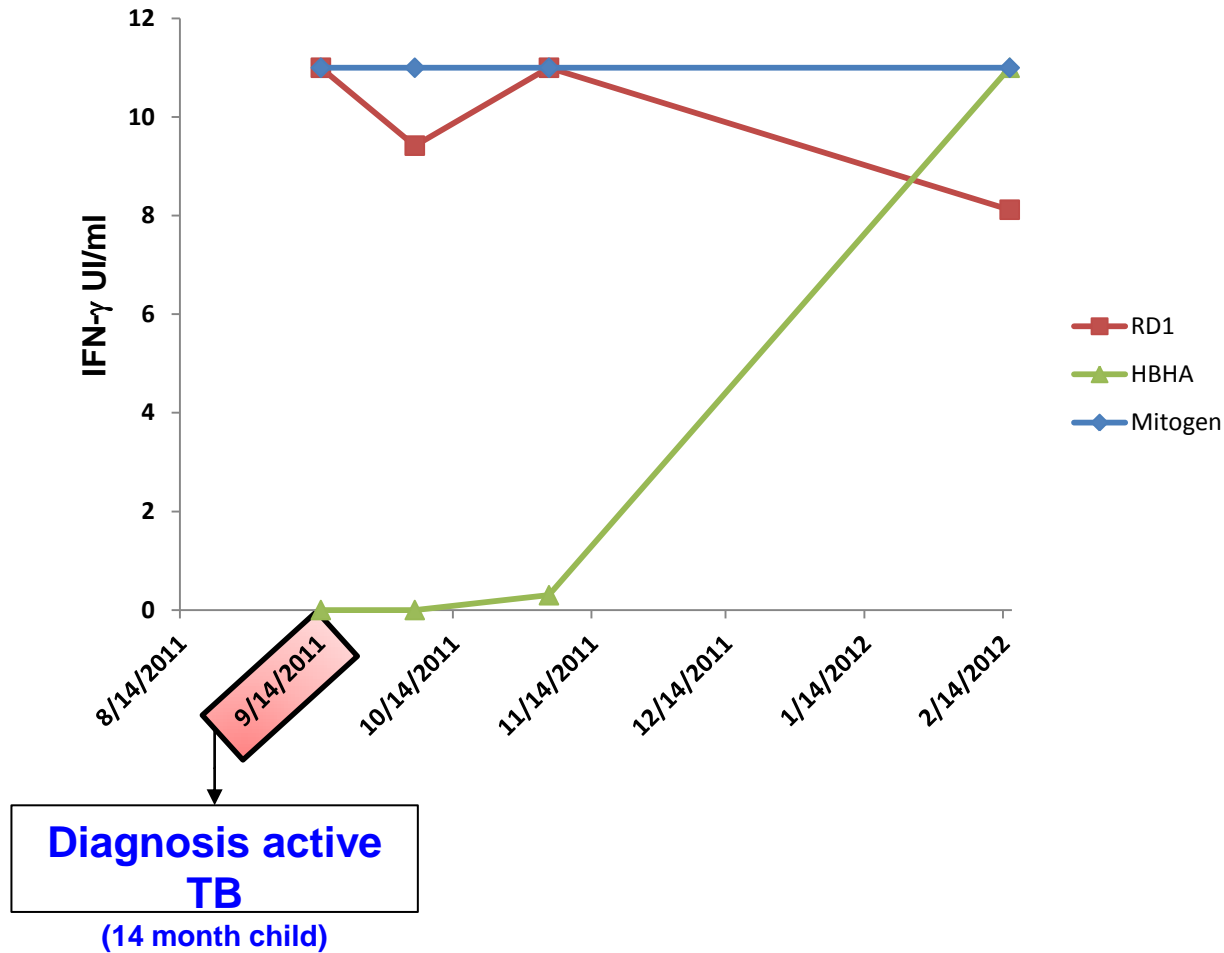
Daniilo Buonsenso, MD, Laura Lancella, MD,† Giovanni Delogu, MD,‡ Andrej Krzysztofiak, MD,† Angela Testa, MD,§ Orazio Ranno, MD,* Pamela D'Alfonso, MD,‡ and Piero Valentini, MD**

TABLE 2. Analyses of QTF Results by Age Groups and Comparison With TST Results

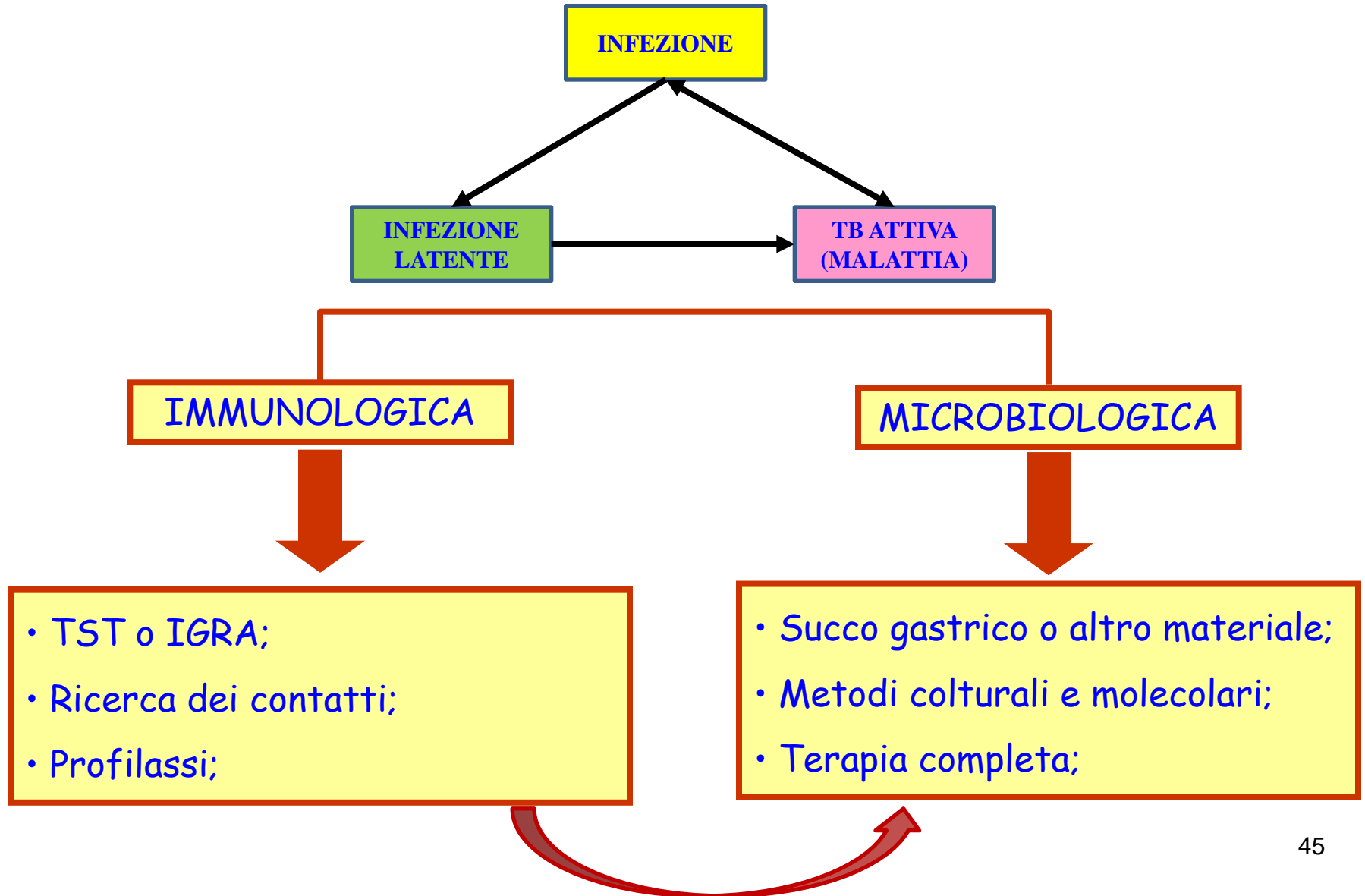
Age	QTF +/Total QTF(%)	TST +/Total TST(%)
0–2 (range: 4 mo to 2 yr)	31/32 (96.9)	23/24 (95.8)
3–5	16/16 (100)	13/14 (92.8)
6–10	5/6 (83.3)	3/5 (60)
11–13	8/8 (100)	4/4 (100)
14–17	4/4 (100)	4/4 (100)

- The results of this study indicate that TST and QTF tests are both effective in children with active TB, although QTF showed a higher sensitivity compared with TST, 97% versus 93.4%, respectively. QTF was also superior to TST when different age groups were considered
- Fifty children (23.4%) were diagnosed with active TB despite no overt sign or symptoms of disease, in line with recent findings that indicate that up to 1 in 4 TB patients is symptom-free at the time of diagnosis. These results further highlight the challenging task of defining active TB and to distinguish it from the latent form of the disease.

T cell responses against *M. tuberculosis* antigens in a child with TB



Diagnosi delle infezioni da *M. tuberculosis*



Evidence to recommendations

- Because of their underdeveloped immune system, children would be more likely to develop active and more serious disease if they had latent infection.
- This risk is greater in children aged under 5 years.
- **The evidence presented that determined the negative predictive values of the tests was of very low quality.**
- **It was agreed that most paediatricians would choose to treat a high-risk child if they had a positive Mantoux test and negative IGT because there was very limited evidence to suggest that a negative IGT could completely exclude infection.**
- The difficulty of phlebotomy and obtaining enough blood in children was discussed, generally in those under five years of age and especially when they are under two years.
- **Indeterminate IGT results occur more frequently in younger children.**
- **The GDG was of the view that IGTs perform less well in younger children.**
- **The group also agreed that careful consideration should be given to high-risk young children, especially those aged under 5 years because false-negative results could have substantial implications.**



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Recommendations and Reports

June 25, 2010 / Vol. 59 / No. RR-5

Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* Infection – United States, 2010

Situations in Which a TST Is Preferred But an IGRA Is Acceptable

- A TST is preferred for testing children aged <5 years. Use of an IGRA in conjunction with TST has been advocated by some experts to increase diagnostic sensitivity in this age group. Recommendations regarding use of IGRAs in children have also been published by the American Academy of Pediatrics (112).

According to the Pediatric Tuberculosis Collaborative Group, preventive therapy is also required for TST negative children <5 years of age who are in close contact with an infectious adult until re-evaluation.

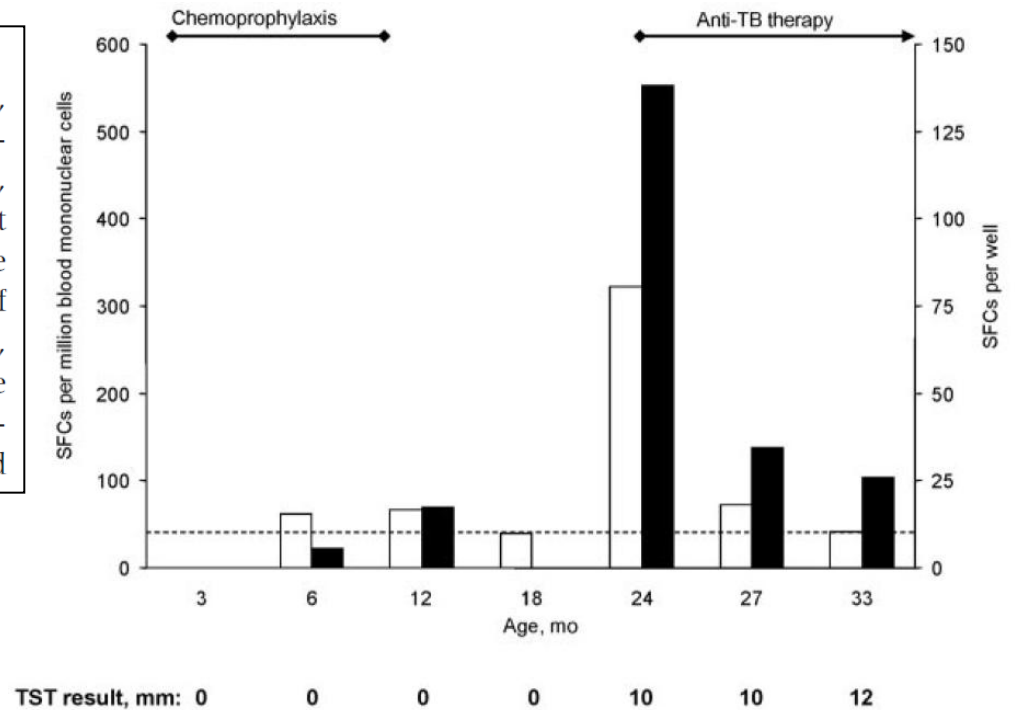
T-Cell-Based Diagnosis of Neonatal Multidrug-Resistant Latent Tuberculosis Infection

Luca Richeldi, MD, PhD^a, Katie Ewer, PhD^b, Monica Losi, PhD^a, Barbara M. Bergamini, MD^a, Kerry Millington, BSc^{b,c}, Leonardo M. Fabbri, MD^a, Ajit Lalvani, FRCP, DM^{b,c}

^aDepartments of Respiratory Disease and Pediatrics, University of Modena and Reggio Emilia, Modena, Italy; ^bTuberculosis Immunology Group, Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom; ^cTuberculosis Immunology Group, Department of Respiratory Medicine, National Heart and Lung Institute, Wright Fleming Institute of Infection and Immunity, Imperial College London, London, United Kingdom

RESULTS

Results of the first ELISpot assay and a repeat TST, performed at 11 weeks of age after 7 weeks of chemoprophylaxis, were negative (Fig 2). At 6 months of age, the ELISpot result was positive and remained positive at a similar level at 12 and 18 months of age, although the TST results remained negative (Fig 2). At 24 months of age, the child presented with a 2-week history of fever, night sweats, and cough. ELISpot results again were positive but with a 10-fold higher response than previously (Fig 2). Responses to phytohemagglutinin and



Sommario

- ✓ I test IGRA mostrano una sensibilità e specificità superiore per la diagnosi di TB attiva anche nei nella popolazione pediatrica;
- ✓ La % di indeterminati è sicuramente molto bassa;
- ✓ E' importante che vengano eseguiti e pubblicati i risultati di studi condotti specificamente nella popolazione pediatrica;
- ✓ Limiti della TST in particolare nella popolazione pediatrica;