Infection, Genetics and Evolution 72 (2019) 78–85

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Paradigm changing evidence that alter tuberculosis perception and detection: Focus on latency

Jeremy Sousa[a,b], Margarida Saraiva[a,b,*]

[a] i3S - Instituto de Investigação e Inovação em Saúde, Porto, Portugal
[b] IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

ARTICLE INFO

Keywords:
Tuberculosis
Latency
Reactivation

ABSTRACT

Tuberculosis remains a devastating disease to Mankind, ranking as the ninth cause of death worldwide. Eliminating tuberculosis as proven much more difficult than once anticipated. In addition to the delay in diagnosis and drug resistance problems that compromise the efficacy of treatment, the enormous reservoir of latently infected individuals continuously feeds the epidemics. However, targeting latency with prophylactic antibiotic administration is not possible at the populational level. Together, these issues call for a better understanding of latency, as well as for a more precise identification of individuals at high risk of reactivation. For this, recent paradigm changing evidence need to be taken into account, most notably, the existence of a tuberculosis spectrum; the genetic diversity of both humans and tuberculosis-causing bacteria; and the changes in the human population that interfere with tuberculosis. Here we discuss latency in the light of these variables and how that understanding can move forward tuberculosis research and elimination.

1. Tuberculosis: a challenging disease

Tuberculosis (TB) has claimed more lives than any other infectious disease and was for a long time considered “the captain of all these men of death” (Rubin, 1995). After the introduction of antibiotic therapy, the expectation was that of TB elimination. However, thus far, TB remains the ninth cause of death world-wide and the first due to infection caused by a single pathogen (WHO: Global tuberculosis control, 2017). In addition to the app. 1.6 million deaths in 2016, 10 million new active TB cases are reported yearly (WHO: Global tuberculosis control, 2017).

Latent TB infection (LTBI) denotes a clinically asymptomatic state, with no radiological or microbiological evidence of disease, where *Mycobacterium tuberculosis* is controlled in a persisting status by the host immune system. Based on recent mathematic modelling, 1.7 billion individuals are latently infected with *M. tuberculosis* (Houben and Dodd, 2016). Therefore, an enormous reservoir of mostly asymptomatic and long-lived individuals exists, creating the conditions for continuously feeding the TB epidemics. Indeed, it is estimated that in low incidence countries 90% of all TB cases result from LTBI reactivation (WHO: Global tuberculosis control, 2017; Vynnycky and Fine, 2000; Cardona, 2016).

The already difficult challenge of devising a strategy to tackle 2 different forms of infection, ie, active and latent TB, is acknowledged to be even more complex. Indeed, latent and active TB are not binary states as once thought, but instead, are now seen as a dynamic spectrum (Fig. 1) varying from latency, to subclinical infection to clinically active disease (Barry 3rd et al., 2009; Ernst, 2012; Getahun, Matteelli, Chaisson, and Raviglione, 2015). Thus, tackling TB requires addressing different positions in this continuous spectrum, considering the particularities of each individual and of each infection. If well succeeded, this major quest will turn the fight against TB in favour of the host. This review focuses on the different aspects that need to be taken into account to this end, discussing exciting new findings in the context of what is limiting our knowledge.

2. The fight against TB: the latency issue

As with any other infectious disease, approaches to decrease TB rely on three main tools: prevention, diagnosis and treatment. These needs are the three main pillars of the End TB Strategy, which aims to reduce the burden of TB worldwide. As excellent reviews are available on these areas, they will be only briefly discussed here. The vaccine in use to

Abbreviations: EGF, epidermal growth factor; IFN, interferon; IGRA, interferon-γ release assay; IL, interleukin; IP-10, interferon-γ-induced protein 10; LTBI, latent tuberculosis infection; MCP, monocyte chemoattractant protein; PET-CT, Positron Emission Tomography and Computerized Tomography; PPD, purified protein derivative; TB, tuberculosis; TST, tuberculin skin test

* Corresponding author at: IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal.
E-mail address: margarida.saraiva@ibmc.up.pt (M. Saraiva).

https://doi.org/10.1016/j.meegid.2018.12.019
Received 3 September 2018; Received in revised form 12 December 2018; Accepted 15 December 2018
Available online 18 December 2018
1567-1348/

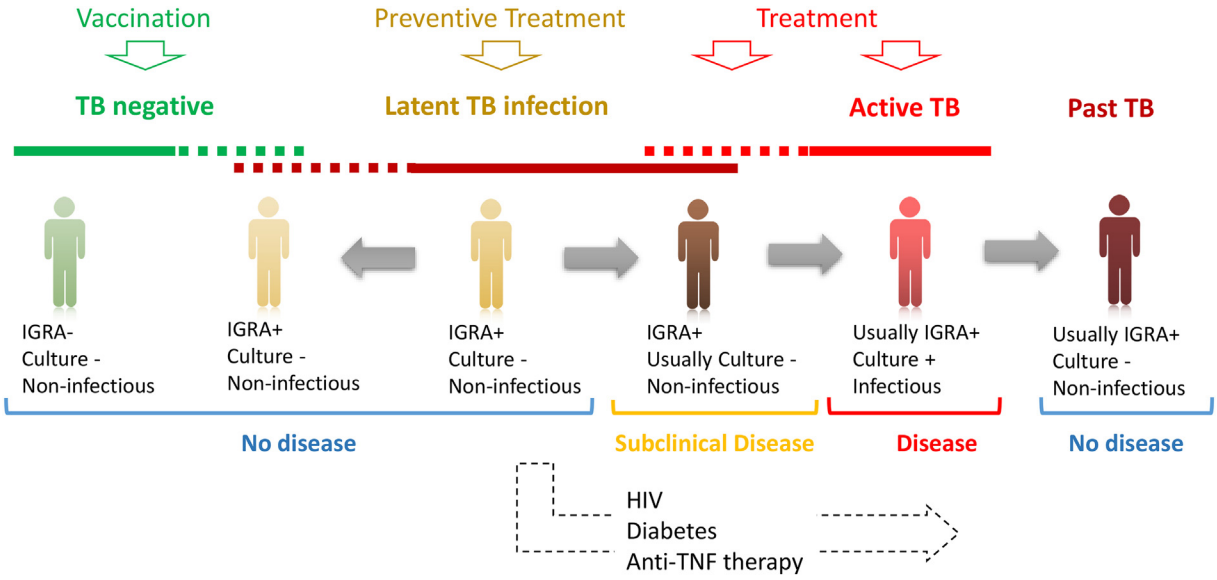

Fig. 1. The different groups of TB. Several outcomes for infection with *M. tuberculosis* have been described. An unknown number of individuals show no signs of cellular responses to previous contact with *M. tuberculosis*, being therefore considered TB negative. Some of these individuals have never been exposed to the bacteria, whilst others may have eliminated it through innate immune mechanisms. An estimated 2 billion individuals control the infection in a latent form, being IGRA + . Of these some appear to eliminate the bacteria, thus becoming TB negative, although no molecular markers are known to identify this process; others progress to active disease through a reactivation step. Failures in the immune system, such as those promoted by HIV, diabetes and immunosuppressive therapies, precipitate the transition between latency and active TB disease. A stage of subclinical disease is now accepted, which can be followed through clinical analysis by PET-CT scan and at the molecular level. Active TB patients show ouvert disease with clinical symptomatology and positive microbiological tests. TB management required by these different groups is different, ranging from vaccination, to preventive therapy to treatment.

protect from *Mycobacterium tuberculosis* infection, *Mycobacterium bovis* BCG, mostly diminishes disease severity in infants and young children, without an evident effect on the incidence of pulmonary TB in adults worldwide (Kaufmann, Evans, and Hanekom, 2015). Several vaccines are being developed and tested in pre-clinical models and clinical trials, but so far none has shown a better protection than BCG (Hatherill, Tait, and McShane, 2016). Our general lack of knowledge on the cellular and molecular bases of protective immune responses to TB, as well as the lack of appropriate pre-clinical models, strongly limit the development of novel vaccines (Ernst, 2018; Karp, Wilson, and Stuart, 2015). Accurate diagnosis is another keystone of TB control as early detection of TB is critical to decrease the period during which active TB patients propagate the infection. Although the wide implementation of GeneXpert has greatly improved TB diagnosis, namely with a unquestionable positive impact in resource limited settings, new diagnostic tools are still needed (Denkinger et al., 2015). TB treatment has classically relied on antibiotherapy, which bares the enormous problem of antimicrobial resistance. Indeed, over 400.000 TB cases per year are due to infection with multi-drug resistant strains, which contributes to a high number of TB-related deaths (WHO: Global tuberculosis control, 2017). Moreover, the time to drug resistance acquisition after the introduction of novel antibiotics (delamanid and bedaquiline) has been shorter than ever before, again risking the emergence of uncontrollable TB (Bloemberg et al., 2015). For all these reasons, the potential of therapies not based in antibiotics is enormous, most notably, of immune-based host-directed therapies (Kaufmann, Dorhoi, Hotchkiss, and Bartenschlager, 2018).

Targeting LTBI is an essential part on fighting TB. Identification and treatment of LTBI has been referred by modelling studies as an effective strategy to reduce TB incidences (Abu-Raddad et al., 2009). So far, preventive therapy appears as the best option to «treat» latency, but risks and benefits of treatment must be carefully balanced for each individual (Fox, Dobler, Marais, and Denholm, 2017). As preventive antibiotherapy is normally prescribed at a dose lower than that used for active TB, and as some of the individuals identified as latently infected are likely to present subclinical disease (Barry 3rd et al., 2009), the development of drug resistant bacteria becomes a risk. Also, the huge number of latently infected individuals makes preventive therapy very difficult to achieve at populational level. Indeed, for a 100% penetrance, about 2 billion people would need therapy with an antibiotic regimen associated with harsh side effects. In all, latency is a well identified problem, preventive therapy is acknowledged to be needed, but not in its current form. Pinpointing latently infected subjects that would really benefit from preventive therapy, ie, those at higher risk of developing active TB, would be a step forward to effectively reduce reactivation.

3. Identification and stratification of latent TB infection: a host centred perspective

There is no gold standard for diagnosing latency. LTBI is defined by the presence of a positive IFN-γ release assay (IGRA) or tuberculin skin test (TST), in the absence of other clinical evidence for active TB, such as lung lesions and *M. tuberculosis* positive cultures (Getahun et al., 2015). Both IGRA and TST measure memory T cell responses to *M. tuberculosis* antigens (Pai and Behr, 2016). IGRA measures IFN-γ production upon stimulation of whole blood with *M. tuberculosis* specific antigens, deleted from the genome of BCG and absent in most environmental mycobacteria (Whitworth, Scott, Connell, Donges, and Lalvani, 2013). On the other hand, TST measures the infiltration of cells to the skin caused by intradermal injection of purified protein derivative (PPD), which is a crude mixture of antigens many of which are shared by *M. tuberculosis*, BCG and several species of environmental mycobacteria. Several limitations have been described for IGRA and TST. Both these tests display a low accuracy in immune-compromised patients; do not distinguish latent from active or subclinical TB, which in TB endemic areas is a major issue; and offer low predicting values for active TB diagnosis (Diel, Loddenkemper, and Nienhaus, 2012; Sester et al., 2014). Furthermore, these tests do not inform on the presence of viable bacilli *in vivo*, as memory T cell responses may persist for years after the infection has been treated. A comparison of IGRA *versus* TST performance for identifying LTBI that progresses to active TB, based on

Table I
Molecular and cellular biosignatures tested as discriminators of LTBI.

Biosignature	Biological sample	Method	Participants	Derivation cohort	Validation cohort	Ref
IP10 PTX3	Plasma	ELISA	220 active TB; 220 household heathy controls; 220 community heathy controls > 15 years old	Republic of Guinea	–	(Rubin, 1995)
IFN-α2, IL-1Ra, sCD40L, IP-10 and VEGF	QuantiFERON Gold stimulated whole blood	Multiplex assay	19 active TB; 57 without active TB 3 months - 5 years old	Cape Town (South Africa)	–	(WHO: Global tuberculosis control, 2017)
IP10	Stimulated Whole Blood	Multiplex assay	127 individuals (7 with active TB) ≤ 17 years old	New York (USA)	–	(Houben and Dodd, 2016)
IP10	Plasma Urine	ELISA	111 children; 33 healthy adult donors 1 month - 16 years old	Kampala (Uganda)	–	(Vynnycky and Fine, 2000)
IP10	QuantiFERON Gold stimulated whole blood	ELISA	17 active TB; 16 LTBI; 16 controls 2 months - 16 years old	London (UK)	–	(Cardona, 2016)
EGF, Fractalkine, IFN-G, IL-4, MCP3	QuantiFERON Gold stimulated whole blood	ELISA	77 HIV negative (37 active TB; 40 healthy donors) and 118 HIV positive (21 active TB; 35 high TB risk; 62 low TB risk) Adults	Rome (Italy)	–	(Barry 3rd et al., 2009)
IP10	QuantiFERON Gold stimulated whole blood	ELISA	66 HIV positive (28 active TB; 38 no active TB) Adults	Chennai and Mumbai (India)	–	(Ernst, 2012)
IP10	Stimulated Whole Blood	Luminex	59 high risk infection and 61 low risk infection (23 as control) < 15 years old	Nigeria	–	(Getahun et al., 2015)
IP10 sTNFR2	Plasma	Multiplex assay ELISA	65 active TB; 34 LTBI; 65 controls Adults	Norway	–	(Kaufmann et al., 2015)
EGF, fractalkine, IFN-g, IL-4, MCP-3 and IP-10	Plasma	Multiplex assay	33 active TB; 30 household contacts Adults	Addis Ababa (Ethiopia)	–	(Hatherill et al., 2016)
IL-6, MCP1, among 30 molecules	Plasma	Multiplex assay ELISA	20 active TB; 12 community controls Adults	Cape Town (South Africa)	–	(Ernst, 2018)
Soluble CD14, MD2	Plasma	ELISA; Immuno Turbido-metric assay	19 active TB; 6 LTBI Adults	Oslo (Norway)	–	(Karp et al., 2015)
EGF, sCD40L, MIP1b, VEGF, TGF-a, IL-1a	QuantiFERON Gold stimulated whole blood	Multiplex assay	23 TB patients; 34 household contacts Adults	Western Cape Province (South Africa)	–	(Denkinger et al., 2015)
Ratio monocytes/ lymphocytes	Peripheral Blood	Full blood counts	1862 HIV-infected adults; 206 of whom developed TB Adults	South Africa	South Africa	(Bloemberg et al., 2015)
Ratio monocytes/ lymphocytes	Peripheral Blood	Full blood counts	1336; 187 of whom developed TB, LTBI or death Infants	South Africa Botswana	–	(Kaufmann et al., 2018)
Ratio monocytes/ lymphocytes	Peripheral Blood	Full blood counts	1202 pre-partum women; 12 of whom developed TB Adults	South Africa, Uganda, Zimbabwe and Tanzania	–	(Abu-Raddad et al., 2009)
Ratio monocytes/ lymphocytes	Peripheral Blood	Full blood counts and FACS	71 active TB; 37 LTBI; 31 healthy donors; 34 cured TB Adults	Rome and Palermo (Italy)	–	(Fox et al., 2017)
Ratio monocytes/ lymphocytes	Peripheral Blood	Full blood counts	296 household contacts > 1 year	Antananarivo (Madagascar)	–	(Pai and Behr, 2016)
CCR6 + CXCR3 + CCR4- T cells	PBMCs	FACS	12 LTBI; 12 control donors Adults	San Diego (California, USA)	–	(Whitworth et al., 2013)
IFN-inducible signature	Whole Blood (RNA)	Microarray	54 (UK) and 53 (South Africa) participants; active TB, LTBI, healthy donors Adults	London (UK)	Cape Town (South Africa)	(Diel et al., 2012)

(*continued on next page*)

Table I (*continued*)

Biosignature	Biological sample	Method	Participants	Derivation cohort	Validation cohort	Ref
IFN-inducible signature	Whole blood (RNA)	Microarray	35 TB patients; 113 controls; 91 other pulmonary diseases Adults	London, Oxford (UK); Paris and Lyon (France)	London, Oxford (UK); Paris and Lyon (France)	(Sester et al., 2014)
Fc gamma receptor	Whole Blood (RNA)	Microarray	33 TB patients; 34 LTBI; 9 controls Adults	Stellenbosch (South Africa)	–	(Auguste et al., 2017)
IFN-inducible signature	PBMCs (RNA)	Microarray	23 TB patients; 23 household controls > 15 years old	Jakarta (Indonesia)	–	(Goletti et al., 2016)
27 transcripts	Whole Blood (RNA)	Microarray	584 participants: active TB; LTBI; other diseases Adults	Cape Town (South Africa); Karonga (Malawi)	Cape Town (South Africa); Karonga (Malawi)	(Azzurri et al., 2005)
Transcripts enriched in "innate immune response," "response to wounding," and "defense response"	Whole Blood (RNA)	Microarray	35 Active TB; 35 LTBI; 39 pneumonia Adults	Colorado and Texas (USA)	African patients	(Chegou et al., 2013)
16-gene transcript signature	Whole Blood (RNA)	RNA-seq	6363; 47 progressors and 107 matched controls Adolescents/Adults	South Africa	South Africa	(Lighter et al., 2009)
4-gene transcript signature	Whole blood	RNA-seq	79 progressors; 328 non-progressors Adults	South Africa; The Gambia	South Africa; The Gambia; Ethiopia	(Petrone et al., 2015)
20-gene TB specific transcript signature	Whole Blood (RNA)	RNA-seq	53 active TB; 50 IGRA-; 49 IGRA+; 9 progressors > 16 years old	Leicester (UK)	Leicester (UK)	(Whittaker et al., 2008)
3-circulating miRNA	Serum	qRT-PCR	43 progressors and 43 non-progressors (South Africa); 11 progressors and 11 non-progressors (Uganda) 151 samples (discovery); 120 samples (validation)	South Africa; Uganda	South Africa; Uganda	(Vanini et al., 2012)
Pulmonary abnormalities	Lung imaging	Chest imaging (x-Ray, FDG-PET/CT scan)	35 LTBI subjects Adults	Cape Town (South Africa)	–	(Goletti et al., 2010a)
FDG-uptake	Lung imaging	Chest imaging (FDG-PET/CT scan)	5 patients with undiagnosed TB Adults	Boston (USA)	–	(Ruhwald et al., 2008)

systematic review of the literature showed inconsistent results, calling to the importance of populational-based studies if an adequate comparison is sought (Auguste et al., 2017). It is thus not surprising that major efforts have been placed in devising alternative methods to better identify LTBI and particularly to stratify the risk of re-activation, as discussed below (Table I).

3.1. Soluble factors and immune cell profiles

Several groups have searched for specific cytokines, chemokines or growth factors indicative of latency, as alternatives to IGRA and TST (Goletti, Petruccioli, Joosten, and Ottenhoff, 2016). An example of this is the chemokine interferon-inducible protein (IP) 10 which has been reported in several studies to be increased in the unstimulated plasma of children and adults with TB (Azzurri et al., 2005; Chegou et al., 2013; Lighter, Rigaud, Huie, Peng, and Pollack, 2009; Petrone et al., 2015; Whittaker, Gordon, and Kampmann, 2008). Similar results have been shown upon stimulation of whole blood (Vanini et al., 2012; Goletti et al., 2010a; Ruhwald et al., 2008). Of note, the performance of IP10 in distinguishing latent from active infection appears to be irrespective of the HIV status (Vanini et al., 2012; Goletti et al., 2010a; Wergeland et al., 2015). These studies placed IP10 as a promising alternative or complementary marker to IFN-γ, which led to the development of different methods to measure it, in different human samples (Goletti et al., 2016). Additionally, plasma concentrations of epidermal growth factor (EGF), fractalkine, IFN-γ, IL-4, monocyte chemoattractant protein (MCP)3 (Mihret et al., 2013), soluble TNF receptor 2 (Wergeland et al., 2015), as well as serum concentrations of IL-6 and MCP1 (Djoba Siawaya, Beyers, van Helden, and Walzl, 2009) have been proposed as means to distinguish LTBI from active TB. Plasma levels of molecules in the toll-like receptor 4 pathway, as soluble CD14 and myeloid differentiation-2 have also been suggested as discriminators of LTBI and active TB (Feruglio, Troseid, Damas, Kvale, and Dyrhol-Riise, 2013). Another study reported the measurement of EGF, sCD40L, MIP-1β, VEGF, TGF-α or IL-1α by multiplex cytokine assay in QuantiFERON TB gold supernatants as a strategy to distinguish active TB from LTBI (Chegou, Black, Kidd, van Helden, and Walzl, 2009).

Association of LTBI with particular immune cell profiles has also been proposed. Most notably, several studies highlight the association of increased monocyte to lymphocyte ratio in the peripheral blood with increased risk of active TB development in HIV/ *M. tuberculosis* co-infected individuals or in children born from HIV-infected mothers (Naranbhai et al., 2014a; Naranbhai et al., 2014b; Naranbhai et al., 2014c). A high monocyte to lymphocyte ratio was also described in active TB as compared to both healthy donors, LTBI subjects and cured TB patients (La Manna et al., 2017). In the same line, in a prospective study comparing TB patients with HIV negative household contacts, the appearance of TB disease symptoms in contacts was significantly associated with an elevated peripheral percentage of blood monocytes (Rakotosamimanana et al., 2015). Of note, monocytes from TB patients appear to differentially express CD64, CD123 and CD152 surface markers when compared to healthy donors, with CD64 expression allowing the best discrimination between active TB patients and LTBI subjects (La Manna et al., 2017). The profiling of *M. tuberculosis*-specific CD4 + T-cells in latently infected individuals *versus* healthy donors highlighted an increased frequency of cells expressing the chemokine signature CCR6 + CXCR3 + CCR4- in the former (Arlehamn et al., 2014). In all, the ratio and characteristics of peripheral blood cellular subpopulations harbor potential as biomarkers of risk of progression to active disease, but more and larger studies are needed before any conclusion is drawn.

3.2. System biology approaches

Seminal studies investigating the transcriptional profile of blood cells from TB patients *versus* healthy uninfected and/or latently *M. tuberculosis*-infected individuals, showed that blood profiling can confirm TB at diagnosis and monitor the overall response to treatment. A transcriptional signature of IFN-inducible 393 gene transcripts in whole blood was initially reported (Berry et al., 2010). Importantly, this signature was found to discriminate patients with active pulmonary TB (in high- and low-incidence TB-burden countries) from healthy controls, patients with other chronic respiratory and systemic conditions, and the majority of latently infected individuals (Berry et al., 2010; Bloom et al., 2013). This signature has been subsequently reported by other studies (Cliff et al., 2013; Joosten, Fletcher, and Ottenhoff, 2013; Maertzdorf et al., 2011; Ottenhoff et al., 2012; Kaforou et al., 2013; Roe et al., 2016; Walter et al., 2016). More recently, the potential value of whole blood transcriptome in predicting differential treatment outcomes was also investigated (Thompson et al., 2017). Thus, system approaches based on whole blood transcriptomics emerged as a platform to discover potential molecular biomarkers discriminating the different TB outcomes (Cliff, Kaufmann, McShane, van Helden, and O'Garra, 2015).

This platform has been also used to search for prospective signatures of risk of TB in healthy individuals (Zak et al., 2016), so that targeted antimicrobial therapy to prevent TB disease could be applied. Zak et al. identified a whole blood 16 gene expression signature through the longitudinal analysis of South African adolescents with LTBI who either developed active TB or remained healthy (Zak et al., 2016). The predictive power of this signature appears to improve for patients close to progression to active TB, which may reflect a status of subclinical disease rather than risk of reactivation from latency in the long term. A follow up to this study subsequently offered a 4 gene PCR-based transcriptomic signature, to predict risk of progression to active TB disease in three African cohorts of recently exposed household contacts of index TB cases (Suliman et al., 2018). As one of the highest risk factors associated with progression to active TB relates to being in recent contact with a patient with active pulmonary TB (Fox, Barry, Britton, and Marks, 2013; Kasaie, Andrews, Kelton, and Dowdy, 2014), identifying household contacts at higher risk of progressing to active TB opens the possibility of envisaging targeted treatment.

Circulating miRNAs differentially expressed in active TB disease *versus* control individuals have been identified over the years (Correia et al., 2017). A recent study prospectively validated a signature of circulating miRNAs for risk of TB disease progression in household contacts in South Africa and Uganda (Duffy et al., 2018). The signature was found to be predictive of household contacts within 6 months of testing, with decreased predictive power found for more distal samples. The authors also found an increase in the circulating miRNA signature close to diagnosis (Duffy et al., 2018), which as discussed above for blood transcriptomic signatures may reflect an immune response to subclinical TB, prior to the development of active disease, rather than latency to active TB progression. It will be interesting to in the future continue the search for additional circulating miRNAs of potential importance in the context of TB progression.

3.3. Lung imaging

LTBI is characterized by the absence of radiological features indicative of disease. Advanced lung imaging is therefore not an option as a diagnostic method for LTBI. However, it is important to refer that the combination of Positron Emission Tomography and Computerized Tomography (PET/CT) led to the identification of subclinical disease in individuals clinically defined as latently infected (Esmail et al., 2016). Consequently, highly sensitive radiological imaging may be useful to identify and treat patients with subclinical TB disease, preventing their transition to active TB, as well as their mistreatment with LTBI chemoprophylaxis. A recent retrospective study also highlighted the potential of PET/CT scans in diagnosing unsuspected TB (Geadas et al., 2018). Of note, analysis of PET/CT imaging was moreover shown to be a useful tool to predict future development of active *versus* latent disease and the propensity for reactivation from a latent state of infection,

in the non-human primate model of infection (White et al., 2017; Lin et al., 2016). This is clearly an area that deserves future research.

3.4. Limitations and challenges

Together, the aforementioned strategies offer important advances towards the development of point-of-care technologies to detect latency and possibly stratify it. Common limitations to these studies are the generally small number of participants included and the lack of validation across different hosts. This not only includes testing the proposed biosignatures in populations with different ethnicities, but also across participants of different ages, in settings of lower TB incidence and in the presence of distinct co-morbidities, such as HIV or diabetes.

Several practical steps have to be overcome before molecular-based tests become a reality, namely to reduce their cost and their dependence on laboratory facilities, so that their application in the field can be implemented. Also, it will be critical to optimize molecular tests to accurately discriminate TB from other diseases. Part of these key issues was addressed in a recent study where a 20 gene reduced TB-specific gene signature was developed following an advanced modular approach and taking into consideration multiple TB cohorts and other diseases (Singhania et al., 2018).

4. Searching for *M. tuberculosis* in latent TB infection: a bacteria centred perspective

The broader view of LTBI encompasses the notion that in latent TB individuals *M. tuberculosis* persists in a dormant, non-replicative form. The prevailing idea has been that during latency *M. tuberculosis* bacilli locates in old fibrotic pulmonary granulomas, metabolically adapted to the low supply of nutrients and hypoxic microenvironment (Parrish, Dick, and Bishai, 1998). Thus, according to this concept, the development of bacteria-based tests to detect latency would target *M. tuberculosis* in a specific location, and possibly at a specific metabolic state. However, more recently, several studies have detected bacterial DNA not only in histologically normal lung tissue specimens from humans and mice during latent infection (Hernandez-Pando et al., 2000; Arriaga, Orozco, Aguilar, Rook, and Hernandez Pando, 2002), but also in extrapulmonary locations (Neyrolles et al., 2006; Barrios-Payan et al., 2012). Therefore, several sites and cell types might constitute reservoirs for persisting *M. tuberculosis*, opening new avenues for latency detection tests based on bacteria DNA recovered from tissues other than the lung. An important consideration for the development of such tests concerns their invasiveness, as for example lung biopsies would not be an option. Inmportantly, recent studies, in mice and Man, showed the persistence of viable *M. tuberculosis* in bone marrow mesenchymal stem cells even after completion of antibiotherapy (Das et al., 2013; Beamer, Major, Das, and Campos-Neto, 2014). Although whether this also happens in latency is not known, these findings raise the possibility to use bone marrow biopsies as specimens to detect presence of *M. tuberculosis* in the absence of clinical symptoms of TB.

In addition to detect *M. tuberculosis* persistence through nucleic acids, recent studies raised the interest on the development of latency tests based on stage-specific *M. tuberculosis* antigens (ref). The antigens encoded by the dormancy of survival regulon (DosR), Rv0081, Rv1733c, Rv1737c, Rv2029c, Rv2031 and Rv2628, have been mostly studied in this context (Corbiere et al., 2012; Goletti et al., 2010b; Wyndham-Thomas et al., 2014; Araujo et al., 2015; Arroyo et al., 2016; Arroyo, Marin, Franken, Ottenhoff, and Barrera, 2018; Bai et al., 2016; Chegou et al., 2012a; Chegou et al., 2012b; Commandeur et al., 2011; Hozumi et al., 2013; Kassa et al., 2012; Mensah et al., 2014). Importantly, all these have shown consistent results in terms of immunogenic potential across different geographic locations. Of note, a recent study has defined the defined the immuno-ORFeome of latent *M. tuberculosis* and investigated its potential for the development of serodiagnostic tools for LTBI (Zhou, Xu, Wu, Cui, and Pan, 2017). However, despite all these studies and promising results, a recent meta-analysis showed still limited evidence on their utility in the context of latency tests (Meier, Jacobsen, Ottenhoff, and Ritz, 2018).

Finally, it is worth mentioning that all these studies are based in the assumption that *M. tuberculosis* exists in a specific dormant state during LTBI. This assumption might however be contested by the fact that existing epidemiological data does not necessarily support such bacterial state during the asymptomatic phase of TB, no matter how prolonged (Behr, Edelstein, and Ramakrishnan, 2018).

5. Host-pathogen diversity and the development of latency stratification tests

The development of latency identification and stratification tests is hampered by the complexity underlying TB, where many different hosts interact with many different bacteria and several distinct outcomes of infection and disease are possible. The diversity of these interactions is much higher than once expected and consequently seldom taken into account. Therefore, the definition of host groups in most TB studies is likely over-simplified and does not reflect real-life scenarios.

On the host side the diversity is enormous, from genetics to disease status to the presence of co-morbidities. In particular, if searching for biomarkers of latency or risk of reactivation, it is critical to distinguish latency from subclinical disease, which is not possible if TST or IGRA tests in the absence of culture positive results are used as proxies of latency. The «TB environment» of the study population is a relevant variable in this sense. TB episodes in individuals previously identified as latently infected are likely associated with reactivation. Thus, follow up studies to identify who reactivated are useful to uncover risk of reactivation biomarkers. However, in high incidence settings it will be very difficult to distinguish latency reactivation from *de novo* infection. Consequently, the search for reactivation biomarkers, that will help to stratify latency, will benefit from low incidence settings, despite being mainly performed so far in high incidence ones. Also, the performance of any biomarkers will need to be validated in individuals with different characteristics, from different ages, to the presence of co-morbidities as HIV or diabetes.

Given the limitation of studying the bacteria during LTBI, it is not surprising that the search for latency biomarkers is overwhelmingly based in decoding the host immune response. However, it is possible that some subjects showing a cellular immune response to *M. tuberculosis*, thus classified as latently infected, may have cleared the bacteria (Pai et al., 2016). Identification of persisting *M. tuberculosis* in latently infected individuals, or of host biomarkers of persistent infection, would be a major step in distinguishing those who still harbor the bacteria from those who have potentially eliminated it, and thus would not benefit from preventive therapy. Evidence of persisting bacteria may be revealed through host responses in light of the recently described phenomenon of trained immunity. Trained immunity is the process by which innate immune cells react to secondary infections, by remaining in a state of enhanced function for a certain amount of time (Netea and van der Meer, 2017). Importantly, trained immunity has been described in response to BCG (Lerm and Netea, 2016) and more recently also in the context of *M. tuberculosis* infection (Joosten et al., 2018). Because trained immunity is not long-lasting it is tempting to speculate that the identification of a trained immunity cellular reprograming reflecting *M. tuberculosis* persistence may be useful as a strategy for latency stratification.

It is important to refer that in either of the aforementioned alternatives (identification of bacterial or host correlates of persisting bacteria in latency), one should not neglect *M. tuberculosis* diversity, a concept that just recently has emerged as relevant in TB. TB is caused by different bacteria distributed in 7 phylo- and geographically related lineages adapted to the human population (Gagneux, 2018). This diversity of the pathogen impacts on several clinical and immunological parameters (Bastos, Osorio, Gagneux, Comas, and Saraiva, 1948) and

the host-pathogen sympatry is broken in the context of immune deregulation, such as HIV co-infection (Fenner et al., 2013). Recent evidence show that the stratification of the bacteria population is important for the interpretation of genetic studies of susceptibility to TB (Bastos et al., 1948). Therefore, variations in the circulating bacteria in the different regions of the world may be an important variable to consider when developing novel immune-based latency detection tests.

6. Conclusion

Exciting advances in our knowledge of host-pathogen interactions in TB and how they modulate the host immune response are opening new avenues towards the development of better TB tools, including latency identification and stratification tests. Several chief aspects now need to be incorporated in fundamental to translational research, most notably to integrate the diversity of hosts, bacteria and settings. This diversity will likely not only require the development and application of different strategies to different geographical locations and individuals, but also an uniformization of protocols between different settings. Altogether, this will move forward our understanding of the cellular and molecular bases for disease susceptibility, thus ultimately contributing to the future development of host-directed therapies to prevent/treat TB in its different forms.

Acknowledgements

We acknowledge funds from Programa Operacional Competitividade e Internacionalização and FEDER through FCT (grant reference: POCI-01-0145-FEDER-028955) and from the British Society for Antimicrobial Research (grant reference: bsac-2017-0156).

References

Abu-Raddad, L.J., Sabatelli, L., Achterberg, J.T., Sugimoto, J.D., Longini Jr., I.M., Dye, C., Halloran, M.E., 2009. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. Proc. Natl. Acad. Sci. U. S. A. 106 (33), 13980–13985.

Araujo, L.S., da Silva, N.B.M., da Silva, R.J., Leung, J.A.M., Mello, F.C.Q., Saad, M.H.F., 2015. Profile of interferon-gamma response to latency-associated and novel in vivo expressed antigens in a cohort of subjects recently exposed to Mycobacterium tuberculosis. Tuberculosis 95 (6), 751–757.

Arlehamn, C.L., Seumois, G., Gerasimova, A., Huang, C., Fu, Z., Yue, X., Sette, A., Vijayanand, P., Peters, B., 2014. Transcriptional profile of tuberculosis antigen-specific T cells reveals novel multifunctional features. J. Immunol. 193 (6), 2931–2940.

Arriaga, A.K., Orozco, E.H., Aguilar, L.D., Rook, G.A., Hernandez Pando, R., 2002. Immunological and pathological comparative analysis between experimental latent tuberculous infection and progressive pulmonary tuberculosis. Clin. Exp. Immunol. 128 (2), 229–237.

Arroyo, L., Rojas, M., Franken, K.L., Ottenhoff, T.H., Barrera, L.F., Multifunctional, T., 2016. Cell Response to DosR and Rpf antigens is associated with protection in long-term mycobacterium tuberculosis-infected individuals in Colombia. Clin. Vaccine Immunol. 23 (10), 813–824.

Arroyo, L., Marin, D., Franken, K., Ottenhoff, T.H.M., Barrera, L.F., 2018. Potential of DosR and Rpf antigens from Mycobacterium tuberculosis to discriminate between latent and active tuberculosis in a tuberculosis endemic population of Medellin Colombia. BMC Infect. Dis. 18 (1), 26.

Auguste, P., Tsertsvadze, A., Pink, J., Court, R., McCarthy, N., Sutcliffe, P., Clarke, A., 2017. Comparing interferon-gamma release assays with tuberculin skin test for identifying latent tuberculosis infection that progresses to active tuberculosis: systematic review and meta-analysis. BMC Infect. Dis. 17 (1), 200.

Azzurri, A., Sow, O.Y., Amedei, A., Bah, B., Diallo, S., Peri, G., Benagiano, M., D'Elios, M.M., Mantovani, A., Del Prete, G., 2005. IFN-gamma-inducible protein 10 and pentraxin 3 plasma levels are tools for monitoring inflammation and disease activity in Mycobacterium tuberculosis infection. Microbes Infect. 7 (1), 1–8.

Bai, X.J., Liang, Y., Yang, Y.R., Feng, J.D., Luo, Z.P., Zhang, J.X., Wu, X.Q., 2016. Potential novel markers to discriminate between active and latent tuberculosis infection in Chinese individuals. Comp. Immunol. Microbiol. Infect. Dis. 44, 8–13.

Barrios-Payan, J., Saqui-Salces, M., Jeyanathan, M., Alcantara-Vazquez, A., Castanon-Arreola, M., Rook, G., Hernandez-Pando, R., 2012. Extrapulmonary locations of mycobacterium tuberculosis DNA during latent infection. J. Infect. Dis. 206 (8), 1194–1205.

Barry 3rd, C.E., Boshoff, H.I., Dartois, V., Dick, T., Ehrt, S., Flynn, J., Schnappinger, D., Wilkinson, R.J., Young, D., 2009. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat. Rev. Microbiol. 7 (12), 845–855.

Bastos, H.N., Osorio, N.S., Gagneux, S., Comas, I., Saraiva, M., 2017. The Troika host-pathogen-extrinsic factors in tuberculosis: modulating inflammation and clinical outcomes. Front. Immunol. 8, 1948.

Beamer, G., Major, S., Das, B., Campos-Neto, A., 2014. Bone marrow mesenchymal stem cells provide an antibiotic-protective niche for persistent viable Mycobacterium tuberculosis that survive antibiotic treatment. Am. J. Pathol. 184 (12), 3170–3175.

Behr, M.A., Edelstein, P.H., Ramakrishnan, L., 2018. Revisiting the timetable of tuberculosis. BMJ 362, k2738.

Berry, M.P., Graham, C.M., McNab, F.W., Xu, Z., Bloch, S.A., Oni, T., Wilkinson, K.A., Banchereau, R., Skinner, J., Wilkinson, R.J., et al., 2010. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466 (7309), 973–977.

Bloemberg, G.V., Keller, P.M., Stucki, D., Trauner, A., Borrell, S., Latshang, T., Coscolla, M., Rothe, T., Homke, R., Ritter, C., et al., 2015. Acquired resistance to bedaquiline and delamanid in therapy for tuberculosis. N. Engl. J. Med. 373 (20), 1986–1988.

Bloom, C.I., Graham, C.M., Berry, M.P., Rozakeas, F., Redford, P.S., Wang, Y., Xu, Z., Wilkinson, K.A., Wilkinson, R.J., Kendrick, Y., et al., 2013. Transcriptional blood signatures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. PLoS One 8 (8), e70630.

Cardona, P.J., 2016. Reactivation or reinfection in adult tuberculosis: is that the question? Int. J. Mycobacteriol. 5 (4), 400–407.

Chegou, N.N., Black, G.F., Kidd, M., van Helden, P.D., Walzl, G., 2009. Host markers in QuantiFERON supernatants differentiate active TB from latent TB infection: preliminary report. BMC Pulm Med. 9, 21.

Chegou, N.N., Black, G.F., Loxton, A.G., Stanley, K., Essone, P.N., Klein, M.R., Parida, S.K., Kaufmann, S.H., Doherty, T.M., Friggen, A.H., et al., 2012a. Potential of novel Mycobacterium tuberculosis infection phase-dependent antigens in the diagnosis of TB disease in a high burden setting. BMC Infect. Dis. 12, 10.

Chegou, N.N., Essone, P.N., Loxton, A.G., Stanley, K., Black, G.F., van der Spuy, G.D., van Helden, P.D., Franken, K.L., Parida, S.K., Klein, M.R., et al., 2012b. Potential of host markers produced by infection phase-dependent antigen-stimulated cells for the diagnosis of tuberculosis in a highly endemic area. PLoS One 7 (6), e38501.

Chegou, N.N., Detjen, A.K., Thiart, L., Walters, E., Mandalakas, A.M., Hesseling, A.C., Walzl, G., 2013. Utility of host markers detected in Quantiferon supernatants for the diagnosis of tuberculosis in children in a high-burden setting. PLoS One 8 (5), e64226.

Cliff, J.M., Lee, J.S., Constantinou, N., Cho, J.E., Clark, T.G., Ronacher, K., King, E.C., Lukey, P.T., Duncan, K., Van Helden, P.D., et al., 2013. Distinct phases of blood gene expression pattern through tuberculosis treatment reflect modulation of the humoral immune response. J. Infect. Dis. 207 (1), 18–29.

Cliff, J.M., Kaufmann, S.H., McShane, H., van Helden, P., O'Garra, A., 2015. The human immune response to tuberculosis and its treatment: a view from the blood. Immunol. Rev. 264 (1), 88–102.

Commandeur, S., Lin, M.Y., van Meijgaarden, K.E., Friggen, A.H., Franken, K.L., Drijfhout, J.W., Korsvold, G.E., Oftung, F., Geluk, A., Ottenhoff, T.H., 2011. Double- and monofunctional CD4(+) and CD8(+) T-cell responses to Mycobacterium tuberculosis DosR antigens and peptides in long-term latently infected individuals. Eur. J. Immunol. 41 (10), 2925–2936.

Corbiere, V., Pottier, G., Bonkain, F., Schepers, K., Verscheure, V., Lecher, S., Doherty, T.M., Locht, C., Mascart, F., 2012. Risk stratification of latent tuberculosis defined by combined interferon gamma release assays. PLoS One 7 (8), e43285.

Correia, C.N., Nalpas, N.C., McLoughlin, K.E., Browne, J.A., Gordon, S.V., MacHugh, D.E., Shaughnessy, R.G., 2017. Circulating microRNAs as potential biomarkers of infectious disease. Front. Immunol. 8, 118.

Das, B., Kashino, S.S., Pulu, I., Kalita, D., Swami, V., Yeger, H., Felsher, D.W., Campos-Neto, A., 2013. CD271(+) bone marrow mesenchymal stem cells may provide a niche for dormant Mycobacterium tuberculosis. Sci. Transl. Med. 5 (170), 170ra113.

Denkinger, C.M., Kik, S.V., Cirillo, D.M., Casenghi, M., Shinnick, T., Weyer, K., Gilpin, C., Boehme, C.C., Schito, M., Kimerling, M., et al., 2015. Defining the needs for next generation assays for tuberculosis. J. Infect. Dis. 211 (Suppl. 2), S29–S38.

Diel, R., Loddenkemper, R., Nienhaus, A., 2012. Predictive value of interferon-gamma release assays and tuberculin skin testing for progression from latent TB infection to disease state: a meta-analysis. Chest 142 (1), 63–75.

Djoba Siawaya, J.F., Beyers, N., van Helden, P., Walzl, G., 2009. Differential cytokine secretion and early treatment response in patients with pulmonary tuberculosis. Clin. Exp. Immunol. 156 (1), 69–77.

Duffy, F.J., Thompson, E., Downing, K., Suliman, S., Mayanja-Kizza, H., Boom, W.H., Thiel, B., Weiner Iii, J., Kaufmann, S.H.E., Dover, D., et al., 2018. A serum circulating miRNA signature for short-term risk of progression to active tuberculosis among household contacts. Front. Immunol. 9, 661.

Ernst, J.D., 2012. The immunological life cycle of tuberculosis. Nat. Rev. Immunol. 12 (8), 581–591.

Ernst, J.D., 2018. Mechanisms of *M. tuberculosis* immune evasion as challenges to TB vaccine design. Cell Host Microbe 24 (1), 34–42.

Esmail, H., Lai, R.P., Lesosky, M., Wilkinson, K.A., Graham, C.M., Coussens, A.K., Oni, T., Warwick, J.M., Said-Hartley, Q., Koegelenberg, C.F., et al., 2016. Characterization of progressive HIV-associated tuberculosis using 2-deoxy-2-[(18)F]fluoro-D-glucose positron emission and computed tomography. Nat. Med. 22 (10), 1090–1093.

Fenner, L., Egger, M., Bodmer, T., Furrer, H., Ballif, M., Battegay, M., Helbling, P., Fehr, J., Gsponer, T., Rieder, H.L., et al., 2013. HIV infection disrupts the sympatric host-pathogen relationship in human tuberculosis. PLoS Genet. 9 (3), e1003318.

Feruglio, S.L., Troseid, M., Damas, J.K., Kvale, D., Dyrhol-Riise, A.M., 2013. Soluble markers of the Toll-like receptor 4 pathway differentiate between active and latent tuberculosis and are associated with treatment responses. PLoS One 8 (7), e69896.

Fox, G.J., Barry, S.E., Britton, W.J., Marks, G.B., 2013. Contact investigation for tuberculosis: a systematic review and meta-analysis. Eur. Respir. J. 41 (1), 140–156.

Fox, G.J., Dobler, C.C., Marais, B.J., Denholm, J.T., 2017. Preventive therapy for latent tuberculosis infection-the promise and the challenges. Int. J. Infect. Dis. 56, 68–76.

Gagneux, S., 2018. Ecology and evolution of Mycobacterium tuberculosis. Nat. Rev. Microbiol. 16 (4), 202–213.
Geadas, C., Acuna-Villaorduna, C., Mercier, G., Kleinman, M.B., Horsburgh, C.R., Ellner, J.J., Jacobson, K.R., 2018. FDG-PET/CT activity leads to the diagnosis of unsuspected TB: a retrospective study. BMC Res. Notes 11 (1), 464.
Getahun, H., Matteelli, A., Chaisson, R.E., Raviglione, M., 2015. Latent *Mycobacterium tuberculosis* infection. N. Engl. J. Med. 372 (22), 2127–2135.
Goletti, D., Raja, A., Syed Ahamed Kabeer, B., Rodrigues, C., Sodha, A., Carrara, S., Vernet, G., Longuet, C., Ippolito, G., Thangaraj, S., et al., 2010a. Is IP-10 an accurate marker for detecting M. tuberculosis-specific response in HIV-infected persons? PLoS One 5 (9), e12577.
Goletti, D., Butera, O., Vanini, V., Lauria, F.N., Lange, C., Franken, K.L., Angeletti, C., Ottenhoff, T.H., Girardi, E., 2010b. Response to Rv2628 latency antigen associates with cured tuberculosis and remote infection. Eur. Respir. J. 36 (1), 135–142.
Goletti, D., Petruccioli, E., Joosten, S.A., Ottenhoff, T.H., 2016. Tuberculosis biomarkers: from diagnosis to protection. Infect Dis. Rep. 8 (2), 6568.
Hatherill, M., Tait, D., McShane, H., 2016. Clinical testing of tuberculosis vaccine candidates. Microbiol. Spectr. (5), 4.
Hernandez-Pando, R., Jeyanathan, M., Mengistu, G., Aguilar, D., Orozco, H., Harboe, M., Rook, G.A., Bjune, G., 2000. Persistence of DNA from Mycobacterium tuberculosis in superficially normal lung tissue during latent infection. Lancet 356 (9248), 2133–2138.
Houben, R.M., Dodd, P.J., 2016. The Global Burden of Latent Tuberculosis Infection: a Re-estimation using Mathematical Modelling. PLoS Med. 13 (10), e1002152.
Hozumi, H., Tsujimura, K., Yamamura, Y., Seto, S., Uchijima, M., Nagata, T., Miwa, S., Hayakawa, H., Fujisawa, T., Hashimoto, D., et al., 2013. Immunogenicity of dormancy-related antigens in individuals infected with Mycobacterium tuberculosis in Japan. Int. J Tuberculosis Lung Dis. 17 (6), 818–824.
Joosten, S.A., Fletcher, H.A., Ottenhoff, T.H., 2013. A helicopter perspective on TB biomarkers: pathway and process based analysis of gene expression data provides new insight into TB pathogenesis. PLoS One 8 (9), e73230.
Joosten, S.A., van Meijgaarden, K.E., Arend, S.M., Prins, C., Oftung, F., Korsvold, G.E., Kik, S.V., Arts, R.J., van Crevel, R., Netea, M.G., et al., 2018. Mycobacterial growth inhibition is associated with trained innate immunity. J. Clin. Invest. 128 (5), 1837–1851.
Kaforou, M., Wright, V.J., Oni, T., French, N., Anderson, S.T., Bangani, N., Banwell, C.M., Brent, A.J., Crampin, A.C., Dockrell, H.M., et al., 2013. Detection of tuberculosis in HIV-infected and -uninfected African adults using whole blood RNA expression signatures: a case-control study. PLoS Med. 10 (10), e1001538.
Karp, C.L., Wilson, C.B., Stuart, L.M., 2015. Tuberculosis vaccines: barriers and prospects on the quest for a transformative tool. Immunol. Rev. 264 (1), 363–381.
Kasaie, P., Andrews, J.R., Kelton, W.D., Dowdy, D.W., 2014. Timing of tuberculosis transmission and the impact of household contact tracing. An agent-based simulation model. Am. J. Respir. Crit. Care Med. 189 (7), 845–852.
Kassa, D., Ran, L., Geberemeskel, W., Tebeje, M., Alemu, A., Selase, A., Tegbaru, B., Franken, K.L., Friggen, A.H., van Meijgaarden, K.E., et al., 2012. Analysis of immune responses against a wide range of *Mycobacterium tuberculosis* antigens in patients with active pulmonary tuberculosis. Clin. Vaccine Immunol. 19 (12), 1907–1915.
Kaufmann, S.H., Evans, T.G., Hanekom, W.A., 2015. Tuberculosis vaccines: time for a global strategy. Sci. Transl. Med. 7 (276), 276fs278.
Kaufmann, S.H.E., Dorhoi, A., Hotchkiss, R.S., Bartenschlager, R., 2018. Host-directed therapies for bacterial and viral infections. Nat. Rev. Drug Discov. 17 (1), 35–56.
La Manna, M.P., Orlando, V., Dieli, F., Di Carlo, P., Cascio, A., Cuzzi, G., Palmieri, F., Goletti, D., Caccamo, N., 2017. Quantitative and qualitative profiles of circulating monocytes may help identifying tuberculosis infection and disease stages. PLoS One 12 (2), e0171358.
Lerm, M., Netea, M.G., 2016. Trained immunity: a new avenue for tuberculosis vaccine development. J. Intern. Med. 279 (4), 337–346.
Lighter, J., Rigaud, M., Huie, M., Peng, C.H., Pollack, H., 2009. Chemokine IP-10: an adjunct marker for latent tuberculosis infection in children. Int. J. Tuberc. Lung Dis. 13 (6), 731–736.
Lin, P.L., Maiello, P., Gideon, H.P., Coleman, M.T., Cadena, A.M., Rodgers, M.A., Gregg, R., O'Malley, M., Tomko, J., Fillmore, D., et al., 2016. PET CT Identifies Reactivation Risk in Cynomolgus Macaques with Latent M. tuberculosis. PLoS Pathog. 12 (7), e1005739.
Maertzdorf, J., Repsilber, D., Parida, S.K., Stanley, K., Roberts, T., Black, G., Walzl, G., Kaufmann, S.H., 2011. Human gene expression profiles of susceptibility and resistance in tuberculosis. Genes Immun. 12 (1), 15–22.
Meier, N.R., Jacobsen, M., Ottenhoff, T.H.M., Ritz, N., 2018. A systematic review on novel *Mycobacterium tuberculosis* antigens and their discriminatory potential for the diagnosis of latent and active tuberculosis. Front. Immunol. 9, 2476.
Mensah, G.I., Addo, K.K., Tetteh, J.A., Sowah, S., Loescher, T., Geldmacher, C., Jackson-Sillah, D., 2014. Cytokine response to selected MTB antigens in Ghanaian TB patients, before and at 2 weeks of anti-TB therapy is characterized by high expression of IFN-gamma and Granzyme B and inter- individual variation. BMC Infect. Dis. 14, 495.
Mihret, A., Bekele, Y., Bobosha, K., Kidd, M., Aseffa, A., Howe, R., Walzl, G., 2013. Plasma cytokines and chemokines differentiate between active disease and non-active tuberculosis infection. J. Inf. Secur. 66 (4), 357–365.
Naranbhai, V., Hill, A.V., Abdool Karim, S.S., Naidoo, K., Abdool Karim, Q., Warimwe, G.M., McShane, H., Fletcher, H., 2014a. Ratio of monocytes to lymphocytes in peripheral blood identifies adults at risk of incident tuberculosis among HIV-infected adults initiating antiretroviral therapy. J. Infect. Dis. 209 (4), 500–509.
Naranbhai, V., Kim, S., Fletcher, H., Cotton, M.F., Violari, A., Mitchell, C., Nachman, S., McSherry, G., McShane, H., Hill, A.V., et al., 2014b. The association between the ratio of monocytes:lymphocytes at age 3 months and risk of tuberculosis (TB) in the first two years of life. BMC Med. 12, 120.
Naranbhai, V., Moodley, D., Chipato, T., Stranix-Chibanda, L., Nakabaiito, C., Kamateeka, M., Musoke, P., Manji, K., George, K., Emel, L.M., et al., 2014c. The association between the ratio of monocytes: lymphocytes and risk of tuberculosis among HIV-infected postpartum women. J. Acquir. Immune Defic. Syndr. 67 (5), 573–575.
Netea, M.G., van der Meer, J.W., 2017. Trained Immunity: an ancient way of remembering. Cell Host Microbe 21 (3), 297–300.
Neyrolles, O., Hernandez-Pando, R., Pietri-Rouxel, F., Fornes, P., Tailleux, L., Barrios Payan, J.A., Pivert, E., Bordat, Y., Aguilar, D., Prevost, M.C., et al., 2006. Is adipose tissue a place for *Mycobacterium tuberculosis* persistence? PLoS One 1, e43.
Ottenhoff, T.H., Dass, R.H., Yang, N., Zhang, M.M., Wong, H.E., Sahiratmadja, E., Khor, C.C., Alisjahbana, B., van Crevel, R., Marzuki, S., et al., 2012. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. PLoS One 7 (9), e45839.
Pai, M., Behr, M., 2016. Latent *Mycobacterium tuberculosis* infection and interferon-gamma release assays. Microbiol. Spectr. 4 (5).
Pai, M., Behr, M.A., Dowdy, D., Dheda, K., Divangahi, M., Boehme, C.C., Ginsberg, A., Swaminathan, S., Spigelman, M., Getahun, H., et al., 2016. Tuberculosis. Nat. Rev. Dis. Primers 2, 16076.
Parrish, N.M., Dick, J.D., Bishai, W.R., 1998. Mechanisms of latency in *Mycobacterium tuberculosis*. Trends Microbiol. 6 (3), 107–112.
Petrone, L., Cannas, A., Aloi, F., Nsubuga, M., Sserumkuma, J., Nazziwa, R.A., Jugheli, L., Lukindo, T., Girardi, E., Reither, K., et al., 2015. Blood or urine IP-10 cannot discriminate between active *Tuberculosis* and respiratory diseases different from Tuberculosis in children. Biomed. Res. Int. 2015, 589471.
Rakotosamimanana, N., Richard, V., Raharimanga, V., Gicquel, B., Doherty, T.M., Zumla, A., Rasolofo Razanamparany, V., 2015. Biomarkers for risk of developing active tuberculosis in contacts of TB patients: a prospective cohort study. Eur. Respir. J. 46 (4), 1095–1103.
Roe, J.K., Thomas, N., Gil, E., Best, K., Tsaliki, E., Morris-Jones, S., Stafford, S., Simpson, N., Witt, K.D., Chain, B., et al., 2016. Blood transcriptomic diagnosis of pulmonary and extrapulmonary tuberculosis. JCI Insight 1 (16), e87238.
Rubin, S.A., 1995. Tuberculosis. Captain of all these men of death. Radiol. Clin. N. Am. 33 (4), 619–639.
Ruhwald, M., Petersen, J., Kofoed, K., Nakaoka, H., Cuevas, L.E., Lawson, L., Squire, S.B., Eugen-Olsen, J., Ravn, P., 2008. Improving T-cell assays for the diagnosis of latent TB infection: potential of a diagnostic test based on IP-10. PLoS One 3 (8), e2858.
Sester, M., van Leth, F., Bruchfeld, J., Bumbacea, D., Cirillo, D.M., Dilektasli, A.G., Dominguez, J., Duarte, R., Ernst, M., Eyuboglu, F.O., et al., 2014. Risk assessment of tuberculosis in immunocompromised patients. A TBNET study. Am. J. Respir. Crit. Care Med. 190 (10), 1168–1176.
Singhania, A., Verma, R., Graham, C.M., Lee, J., Tran, T., Richardson, M., Lecine, P., Leissner, P., Berry, M.P.R., Wilkinson, R.J., et al., 2018. A modular transcriptional signature identifies phenotypic heterogeneity of human tuberculosis infection. Nat. Commun. 9 (1), 2308.
Suliman, S., Thompson, E., Sutherland, J., Weiner Rd, J., Ota, M.O.C., Shankar, S., Penn-Nicholson, A., Thiel, B., Erasmus, M., Maertzdorf, J., et al., 2018. Four-gene Pan-African blood signature predicts progression to tuberculosis. Am. J. Respir. Crit. Care Med. 197 (Epub ahead of print).
Thompson, E.G., Du, Y., Malherbe, S.T., Shankar, S., Braun, J., Valvo, J., Ronacher, K., Tromp, G., Tabb, D.L., Alland, D., et al., 2017. Host blood RNA signatures predict the outcome of tuberculosis treatment. Tuberculosis (Edinb) 107, 48–58.
Vanini, V., Petruccioli, E., Gioia, C., Cuzzi, G., Orchi, N., Rianda, A., Alba, L., Giancola, M.L., Conte, A., Schinina, V., et al., 2012. IP-10 is an additional marker for tuberculosis (TB) detection in HIV-infected persons in a low-TB endemic country. J. Inf. Secur. 65 (1), 49–59.
Vynnycky, E., Fine, P.E., 2000. Lifetime risks, incubation period, and serial interval of tuberculosis. Am. J. Epidemiol. 152 (3), 247–263.
Walter, N.D., Miller, M.A., Vasquez, J., Weiner, M., Chapman, A., Engle, M., Higgins, M., Quinones, A.M., Rosselli, V., Canono, E., et al., 2016. Blood transcriptional biomarkers for active tuberculosis among patients in the United States: a Case-Control Study with Systematic Cross-Classifier Evaluation. J. Clin. Microbiol. 54 (2), 274–282.
Wergeland, I., Pullar, N., Assmus, J., Ueland, T., Tonby, K., Feruglio, S., Kvale, D., Damas, J.K., Aukrust, P., Mollnes, T.E., et al., 2015. IP-10 differentiates between active and latent tuberculosis irrespective of HIV status and declines during therapy. J. Inf. Secur. 70 (4), 381–391.
White, A.G., Maiello, P., Coleman, M.T., Tomko, J.A., Frye, L.J., Scanga, C.A., Lin, P.L., Flynn, J.L., 2017. Analysis of 18FDG PET/CT imaging as a tool for studying *Mycobacterium tuberculosis* infection and treatment in non-human primates. J. Vis. Exp.(127).
Whittaker, E., Gordon, A., Kampmann, B., 2008. Is IP-10 a better biomarker for active and latent tuberculosis in children than IFNgamma? PLoS One 3 (12), e3901.
Whitworth, H.S., Scott, M., Connell, D.W., Donges, B., Lalvani, A., 2013. IGRAs–the gateway to T cell based TB diagnosis. Methods 61 (1), 52–62.
WHO, 2017. Global Tuberculosis Control.
Wyndham-Thomas, C., Corbiere, V., Dirix, V., Smits, K., Domont, F., Libin, M., Loyens, M., Locht, C., Mascart, F., 2014. Key role of effector memory CD4+ T lymphocytes in a short-incubation heparin-binding hemagglutinin gamma interferon release assay for the detection of latent tuberculosis. Clin. Vaccine Immunol. 21 (3), 321–328.
Zak, D.E., Penn-Nicholson, A., Scriba, T.J., Thompson, E., Suliman, S., Amon, L.M., Mahomed, H., Erasmus, M., Whatney, W., Hussey, G.D., et al., 2016. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet 387 (10035), 2312–2322.
Zhou, F., Xu, X., Wu, S., Cui, X., Pan, W., 2017. ORFeome-based identification of biomarkers for serodiagnosis of Mycobacterium tuberculosis latent infection. BMC Infect. Dis. 17 (1), 793.