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The prognosis of latent tuberculosis: can disease be predicted?

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In humans, *Mycobacterium tuberculosis* persists for long periods in a clinically latent state, creating a huge reservoir of 'silent' tuberculosis (TB) (roughly one-third of the global population) from which new cases continually arise. A prognostic marker for active TB would enable targeted treatment of the small fraction of infected individuals who are most at risk of developing contagious TB, contributing greatly to TB control efforts. Here, we propose that TB-specific interferon-γ release assays might be useful for identifying individuals with progressive infections who are likely to develop the disease. This might provide an unprecedented advantage for TB control, namely targeted preventive therapy for individuals who are most at risk of developing active contagious TB.

The global challenge of latent tuberculosis

Tuberculosis (TB) is responsible for 2–3 million deaths every year and, probably, 30 times as many infections [1]. This creates a huge reservoir of untreated latent TB infection (LTBI), which can reactivate later in life, and represents a major source of disease [1]. Despite the existence of effective treatment regimens, control of TB is complicated by the chronic nature of the infection. The fact that only 5–10% of recently exposed individuals develop clinically active TB in the first two years after exposure, together with the often casual nature of exposure, makes diagnosis of LTBI among recently exposed and potentially infected individuals extremely difficult. For many decades, different versions of the tuberculin skin test (TST) have been used, despite widespread recognition of its low specificity for TB and its inability to distinguish reliably individuals infected with *Mycobacterium tuberculosis* from those vaccinated with Bacillus Calmette-Guerin (BCG). Only recently, specific tests have emerged that replace the TST for the diagnosis of LTBI. These are based on the release of interferon-γ (IFN-γ) in blood samples after re-stimulation *in vitro* with *M. tuberculosis*-specific antigens such as the 6 kDa early secreted antigenic target (ESAT-6) and culture filtrate protein 10 kDa (CFP-10) [2]. At present, these IFN-γ release assays (IGRAs) are used to identify infected individuals after exposure to *M. tuberculosis* (MTB) and, in developed countries, they are now considered a useful diagnostic tool [3–5].

Here, we discuss emerging evidence indicating that T-cell responses to these TB-specific antigens correlate with bacterial replication, offering potential prediction of progression of infection and disease. We hypothesize that, in addition to the current low cut-off values used in these tests for sensitive identification of infected individuals, it might be possible to establish an incipient-disease cut-off value or conversion that identifies individuals in advanced stages of infections who are likely to develop the disease (Box 1). This might provide an unprecedented advantage for TB control, namely targeted preventive therapy of individuals who are most at risk of developing active contagious disease.

TB infection, immune responses and diagnosis

The study of the immune response to TB is an active research area; it is not within the scope of this article to review the extensive literature on this rapidly growing field (for a review, see [6]). However, it is important to understand which component(s) of the immune response can be used to diagnose the different stages of TB infection and disease. MTB is an intracellular pathogen that resides mainly within macrophages and is able to survive for many years in an intracellular habitat in a slowly-replicating or non-replicating state that is induced by host immune responses and fibrotic encapsulation. Recently, there has been a breakthrough in the understanding of the adaptation of MTB to the hostile intracellular environment of the immune host [7]. MTB responds to the host immune system with dynamic transcriptional changes of a subset of its 4000 genes. Mimicking growth conditions *in vivo* by O_2 depletion, nutrient starvation or nitric oxide (NO) addition has led to the identification of several MTB genes, the expression of which is rapidly altered to enable intracellular survival [e.g. the dormancy (DosR) regulon, which consists of 48 genes] [8].

It has long been recognized that cell-mediated immune (CMI) responses predominate in TB infection; specifically, a type-1 T-cell response that is characterized by production of IFN-γ and interleukin-2. During the initial phase of infection, when mycobacteria are present almost exclusively within macrophages, little if any free unprocessed antigen leaves the macrophage and is available to stimulate a humoral immune response. Antigens that are secreted by the replicating mycobacteria are clearly presented by infected antigen-presenting cells, as indicated by the rapid induction of a strong CMI response [9]. However, recent data suggest that the molecules that are encoded by

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Box 1. What is latency?

A latent infection can be defined as one which is 'subclinical' – that is, an infection without noticeable symptoms. However, for the prognosis of *M. tuberculosis* infection, it is important to distinguish between a recent infection, where symptoms have not yet developed, a long-term latent infection, where the host successfully contains the pathogen, and an advanced stage of infection (here defined as incipient disease) that leads to clinical disease.

some of the genes in the DosR regulon are recognized later, when a long-term latent infection is established [10,11].

Consequently, measurement of CMI rather than antibody responses provides a sensitive way to detect early TB infection (Figure 1). However, if an individual develops active TB and the bacterial and antigenic load increases, a robust antibody response [12,13] and measurable levels of free antigen (e.g. in sputum or urine) can be detected [14,15]. Therefore, the most successful approach so far in the development of an effective new diagnostic test for TB infection has been the measurement of *M. tuberculosis*-specific type-1 T-cell responses, for which IFN-γ is an appropriate marker.

The tuberculin skin test

Until recently, the TST, which is an interdermal injection of purified protein derivative (PPD), was the only tool for detecting LTBI. The TST measures a delayed-type hypersensitivity reaction based on immunological recognition of mycobacterial antigens in exposed individuals, and is a simple and inexpensive test. However, as PPD contains a poorly defined mixture of mycobacterial antigens, many factors other than TB infection influence the outcome of the test, including BCG vaccination and prior exposure of the subject to non-tuberculous mycobacteria [16,17]. In countries in which BCG is widely used, as many as 60% to 90% of people with low risk of MTB infection can be identified as false positive by the TST [18,19], although this might not be true in all settings, such as in Gambian children [20]. Different TST cut-off values and different tuberculin PPD preparations and concentrations have been used to improve the specificity of the test, taking into consideration BCG-vaccination status, exposure to environmental mycobacteria and likelihood of infection [21,22]. Although the TST has its shortcomings, it is clinically useful and a positive TST remains the most important criteria for triggering preventive therapy among contacts. Interestingly, recent studies performed in Malaysia and Hong Kong demonstrated that, even in a region with widespread TST positivity due to high BCG coverage and latent TB, there is a clear correlation between the size of the TST-induced induration and subsequent development of active TB [23,24].

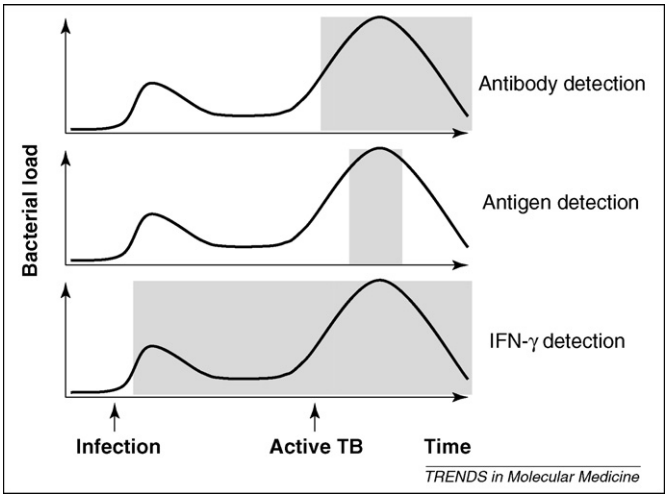

Figure 1. Schematic representation of the immune response of individuals during the course of infection. The shade areas illustrate when it is possible to detect a response using the given test. As early as two weeks after infection with *M. tuberculosis*, a cell-mediated immunity response can be measured. This response is associated with both delayed type hypersensitivity (DTH) responses, as measured by the Mantoux test, and production of type 1 T-cell cytokines, IFN-γ, and often correlates with a temporary arrest or delay of bacterial growth. This response is maintained throughout the course of the infection, but it can wane in individuals who develop very severe TB. Mycobacterial load remains low during the early phase of infection and is therefore not accessible for antigen-detection assays. In individuals who develop acute disease, mycobacterial load and soluble antigen increase. Similarly, *M. tuberculosis*-specific antibody responses are usually undetectable during the early phase of infection, but develop as the infection progresses to active TB.

Discovery and characteristics of *M. tuberculosis*-specific antigens

The major breakthrough in the search for novel specific diagnostic reagents came with the identification of the genetic differences between the *Mycobacterium bovis* BCG vaccine strain, virulent *M. bovis* and *M. tuberculosis* [25,26]. During the attenuation process leading to the various BCG strains used worldwide, several genetic segments are lost (the so-called regions of difference, RDs) from the original virulent *M. bovis* strain. The identification and use of antigens encoded by these RDs for diagnosis of TB infection has previously been reviewed [27]. The two that are most well-characterized, ESAT-6 and CFP-10, are encoded by the RD1 region of the *M. tuberculosis* genome, which is deleted in all BCG vaccine strains. The genes encoding ESAT-6 and CFP-10 are also absent in most non-tuberculous mycobacteria, with the exception of the opportunistic pathogens *Mycobacterium szulgai*, *Mycobacterium marinum* and *Mycobacterium kansasii*. Importantly, both ESAT-6 and CFP-10 are secreted from replicating bacteria *in vitro* [28] and *in vivo* [29], and the secretion of these molecules correlate with the virulence of different genetically modified strains of *M. tuberculosis* [30].

The novel specific tests – upgrading diagnosis of TB from induration to IFN-γ

With the identification of the antigens ESAT-6 and CFP-10, the way was paved for the development of specific *in vitro* diagnostic tests, and several test formats that measure IFN-γ production have been evaluated over recent years [2,31]. Two standardized diagnostic kits that contain the ESAT-6 and CFP-10 antigens are now on the market: the QuantiFERON-TB GOLD (QFT) test (Cellestis Limited, Carnegie, Victoria, Australia) and the T-SPOT.TB assay (Oxford Immunotec, Oxford, UK). Both assays are useful to detect LTBI. Specificity is very high [32–34] and, as expected, BCG vaccination is not a confounding factor for these tests [35]. Assessment of the sensitivity of these tests for LTBI has been complicated

by the lack of a gold standard. However, through screening contacts of active TB cases, in which the association between test results and the level of exposure of contacts to the index case(s) is recorded, the current consensus is that both tests detect individuals affected by LTBI with a sensitivity at least as high as the TST, and the response correlates better with the level of exposure than does the TST [4,19,36–38]. Several recent studies have directly compared the performance of the TST with that of the two novel tests [34,39]. In Europe, USA and Japan, where these tests have entered the market, the value of this approach in contact tracing has rapidly become apparent [36,40]. In the USA, the Centers for Disease Control (CDC) recommends the use of the QFT test instead of the TST for contact investigation, evaluation of recent immigrants and serial testing for infection control [5]. In the UK, the National Institute of Health and Clinical Excellence (NICE) has recommended IFN-γ testing for confirming a positive TST or in individuals for whom the Mantoux test might be less reliable (www.nice.org.uk/page.aspx?o=CG033NICEguideline).

However, to achieve maximum sensitivity for infection, the two test kits have established their optimal cut-off values at the lowest possible level [32,41]. The QFT cut-off value is based on receiver operating characteristic (ROC) curve analyses in which specificity in non-exposed healthy community controls (from a low endemic setting) is compared with sensitivity in patients with active pulmonary TB [18,42]. Indeed, in the study of Mori *et al.* [18], where community controls have no real exposure to MTB, and therefore have almost no risk for latent infection, the specificity is very high. Essentially, positive results are found only in TB patients. Thus, the cut-off value for what defines a positive test can be set very low. This has resulted in diagnostic tests that are designed to detect the maximum number of individuals with an MTB infection. However, the current versions of the tests do not provide information on which individuals are more likely to develop the disease.

Correlation between T-cell responses to ESAT-6 and CFP-10, and disease progression in animal models

Although clinical outcome (i.e. development of active disease) is the only feasible read-out in human investigations of the predictive value of ESAT-6, animal studies provide the advantage of direct assessment of disease status and bacterial replication. In animal models, CMI responses to both ESAT-6 and crude culture filtrate correlate closely with bacterial replication *in vivo* and with the progression of disease. This was first demonstrated by comparing T-cell proliferative responses with culture filtrate antigens and bacterial numbers at different time points post-infection [9], and later by studying the dynamics of T-cell responses that are specific for ESAT-6 in mice infected by MTB [43] or genetically modified strains [30]. It might seem contradictory that a high ESAT-6 response correlates with ongoing bacterial replication, as generating a strong IFN-γ response to ESAT-6 by vaccination leads to efficient control of bacterial replication in a subsequent infection (Box 2). The predictive value of IFN-γ response to ESAT-6 has been recently addressed in a study that monitored the efficacy of novel experimental vaccines in the aerosol mouse model [44]. This study demonstrated a close correlation between ESAT-6-stimulated IFN-γ levels two weeks post-infection and the subsequent bacterial numbers in the lungs when necropsied at week 6 post-infection [44]. Furthermore, in cattle infected with *M. bovis*, high IFN-γ responses to ESAT-6 early in the infection predicted the animals that later developed progressive disease or that were later (at necropsy) positive for *M. bovis* – something that the response to other antigens did not do [45]. Vordermeier *et al.* [46] later observed a direct correlation between the quantity of IFN-γ secreted in response to ESAT-6 and the degree of pathology in cattle infected with *M. bovis*. The close correlation between ESAT-6 responses and progression of disease has subsequently been confirmed in two more studies in cattle [47,48] and in a recent study in non-human primates [49]. A crucial and often overlooked aspect in all these studies is that it is not the presence of ESAT-6-stimulated IFN-γ that correlates with the outcome: cells from all infected animals produce IFN-γ in response to re-stimulation with ESAT-6. It is the amount of IFN-γ produced (or the number of antigen-specific cells) in response to ESAT-6 that correlates with the ability to restrict bacterial growth.

Box 2. The role of IFN-γ

It might seem paradoxical that IFN-γ, an essential T-cell helper 1 (Th1) cytokine for host defense against mycobacteria, can serve as a marker for failing immunity. However, the symptoms in TB are immunopathological, and the same inflammatory cytokines that can eliminate the bacteria are also essential for its transmission; without tissue destruction and cavitation, the bacteria cannot reach the lumen of the bronchioles to be spread in the sputum. In most cases, immune activation (characterized by IFN-γ production) leads to the suppression of infection and the subsequent downregulation of the immune response. In some individuals, however, the bacteria survive by manipulating the host's immune response [62]. In this case, the immune response, which is directly stimulated by the bacteria, increases in magnitude as the bacteria increase in numbers and, eventually, it might become pathological rather than protective. Similarly, in animal models a certain level of vaccine that promotes an IFN-γ response to ESAT-6 can be protective, whereas in non-vaccinated animals that do not control the early stages of infection the magnitude of the ESAT-6 response after infection correlates with bacterial replication.

Correlation between T-cell responses to ESAT-6 and CFP-10, and clinical outcome

Because of a carefully established cut-off value for ESAT-6- and CFP-10-stimulated IFN-γ responses, the IGRAs are becoming an established standard for the diagnosis of MTB infection. It is therefore relevant to re-address some of the intriguing first reported observations made when measuring ESAT-6 immune responses in TB-endemic regions. The first evaluation of ESAT-6 recognition in close contacts such as household contacts to a sputum-positive TB patient in a TB-endemic region showed that most contacts (and many of the community controls) were positive in agreement with the cut-off value established for the commercially available IGRAs (~18 pg/ml). However, within this positive group, subjects can roughly be divided into three distinct subgroups (i.e. low, moderate and high responses) with markedly different

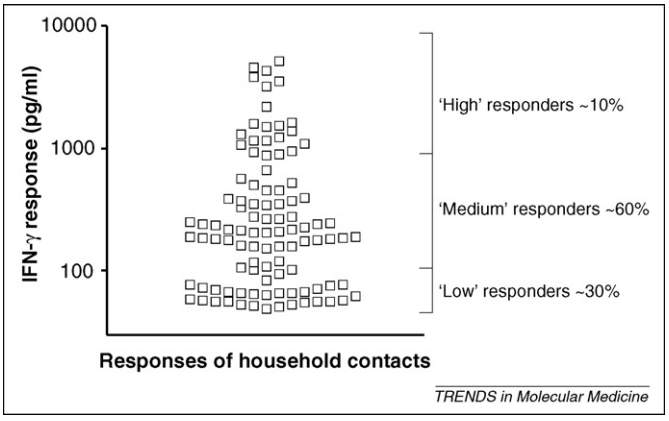

Figure 2. Distribution of ESAT-6 responses among TB contacts in high endemic settings (e.g. Ethiopia). This figure shows that the immune responses (assessed here as IFN-γ production) to ESAT-6 after exposure to infectious TB patients are not normally distributed. The subjects are grouped into low (<100 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].

ESAT-6 response levels [50]. Recently, grouping of response levels has been further substantiated by studies of ESAT-6- and CFP-10-stimulated IFN-γ responses in Ethiopia [51,52] (Figure 2). An overall assessment of the data suggests that ~30% of the contacts had low IFN-γ responses in the range 0–100 pg/ml (100 pg/ml was the cut-off value for a positive response for the assay system used in this study), whereas ~10% had exceedingly high levels of IFN-γ (>1000 pg/ml). In between these two populations lie ~60% of the contacts, covering a large range of test positive responses (Figure 2). These different ESAT-6 response levels predicted a different outcome of infection as suggested by a study that, although based on small numbers, showed that very strong ESAT-6 responses shortly after exposure to MTB correlated with subsequent development of disease [51]. In these recently exposed, healthy contacts, high levels of IFN-γ in response to ESAT-6 (in this study, ~5000 pg/ml) were associated with a tenfold increased risk of subsequently developing clinical TB in the 1–2 years immediately after exposure compared with those healthy contacts from the same households with a low IFN-γ response to ESAT-6. The overall correlation of ESAT-6-specific IFN-γ levels and stage of infection is strongly supported by recent epidemiological investigations, indicating a correlation between the infectious dose to which contacts have been exposed and the magnitude of their response to PPD and ESAT-6 *in vitro*, although the latter was only a trend (p=0.08) [53].

Recent longitudinal studies from TB endemic countries provide further support to our hypothesis. In a study conducted in India, Pai *et al.* [54] showed that highly exposed healthcare workers (HCWs) who had TST conversions in India had massive increases in IFN-γ responses to ESAT-6 and CFP-10 (measured using the QFT in tube assay). The QFT assay successfully detected all cases of TST conversion, and every HCW who had a large increase in TST induration had a huge increase in IFN-γ response, which was significantly higher than the diagnostic cut-off point of 0.35 IU/ml. There were similar findings (using ELISPOT) in recent longitudinal studies of nursing students from Zimbabwe [55] and household contacts from Uganda [56]. Thus, it is plausible that individuals with recent exposure to TB have vigorous increases in T-cell responses, probably due to active bacterial replication. Because it is well documented that individuals with recent TST conversions have a high probability to develop the active disease [57], it is plausible that strong increases in IFN-γ responses after recent exposure might predict progression towards active disease.

Therefore, on the basis of these studies, we hypothesize that high and/or rising levels of IFN-γ produced in response to ESAT-6 by T cells from recently TB-infected individuals signal incipient disease and, thus, might serve as a prognostic marker for subsequent development of overt disease (Figure 3). This hypothesis is in agreement with the observation that ESAT-6-stimulated IFN-γ response levels decline significantly during successful therapy (when both bacterial load and risk of disease also decline) [58–60].

The potential use of IFN-γ assay as a prognostic test

Most TB cases are concentrated in resource-poor countries where the incidence of active TB represents the tip of the iceberg compared with the immense pool of LTBI. At present, most TB control efforts in these countries are devoted to the identification and chemotherapy of patients with active TB. This makes sense in a resource-poor setting, because these cases are already infectious and, thus, the major source of new cases. However, even in these settings, treating LTBI before the development of active disease also makes sense both economically and in terms of public health, especially in high-risk groups. Unfortunately, the immense numbers of LTBI-infected individuals makes identification and treatment almost impossible using existing approaches and resources. Nevertheless, what might be possible in these countries is a better targeted approach that aims to identify and treat those individuals with incipient disease. As suggested above, these would most likely be identified via their exceedingly high and/or rising levels of ESAT-6-specific IFN-γ (Figure 3). Similar to the TST, in which a 5 mm result is considered positive in individuals without confounding factors (such as vaccination) in many developed countries, but considered negligible in African and most Asian countries, cut-off values can be meaningfully discussed only in a specific context (Box 3). The major practical problem is setting appropriate cut-off values and the challenge is not to identify positive responses *per se*, but to establish a cut-off point within the positive category that predicts subsequent development of active TB. Furthermore, a high IFN-γ response measured 10–30 years after exposure in healthy individuals might not pose the same risk as a high IFN-γ response measured 2–3 months after exposure. Therefore, in addition to identifying an absolute cut-off level for incipient disease, a certain increase in the IFN-γ response among recently exposed individuals (conversion) might be a strong indicator of bacterial replication and, therefore, a predictor of progression to active disease [54]. Prognosis based on conversion will be most helpful in situations in which serial testing is usually done (e.g. annual screening of HCWs as part of TB-infection control programs). A completely separate issue is the utility of this test in individuals co-infected with HIV. Depending on the

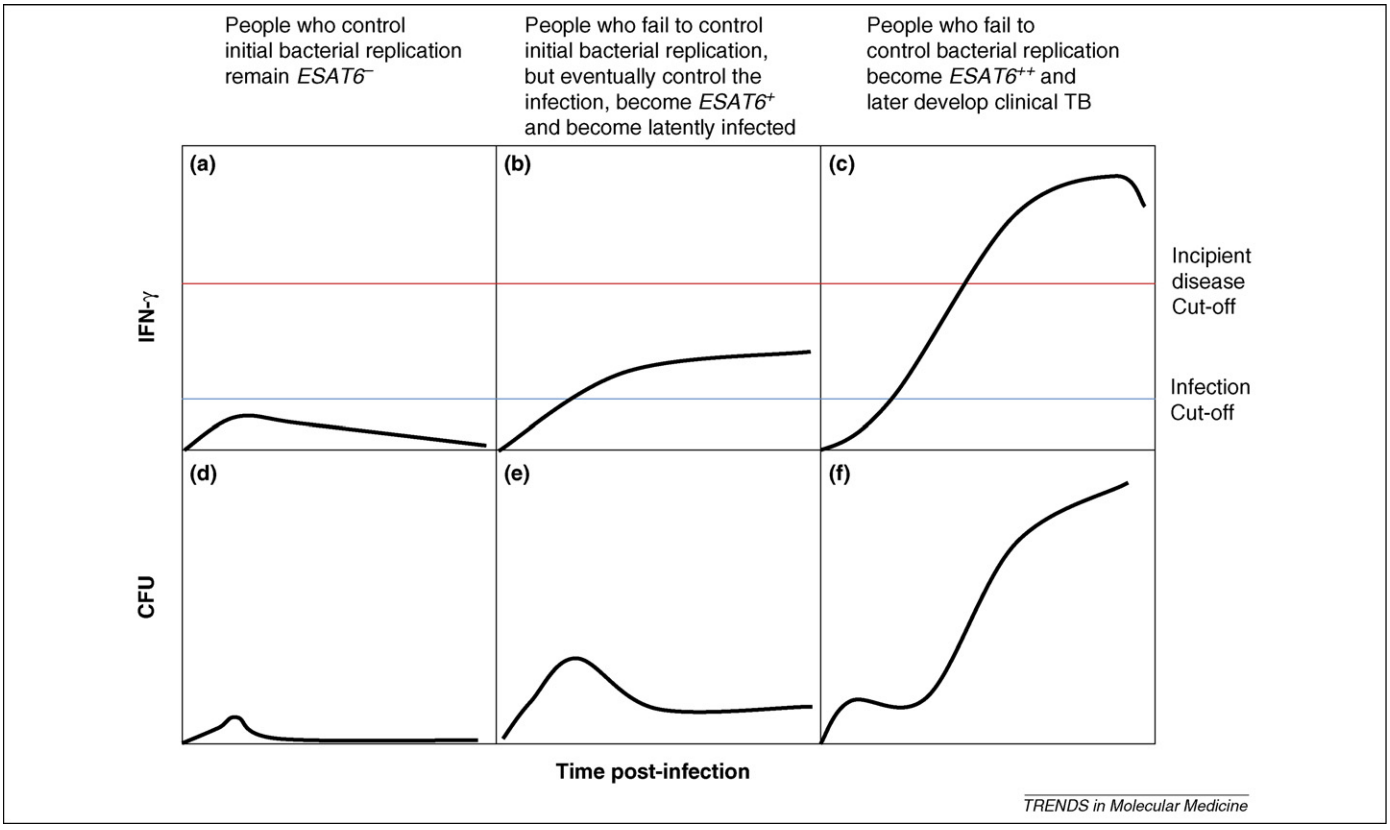

Figure 3. Schematic representation showing the postulated relationship between bacterial load, measured as colony forming units (CFUs), ESAT-6 response levels and clinical outcome. Initial infection might be controlled at its onset with minimal bacterial replication and induction of ESAT-6 responses **(a,d)**. However, in most cases, initial bacterial replication reaches a point where it induces an ESAT-6-specific IFN-γ response to increase above the established cut-off value (infection threshold), enabling the identification of an individual as latently infected **(b,e)**. In most cases, individuals control the infection, resulting in latent infection, but some develop acute TB associated with progressive bacterial replication. This is accompanied by increasing ESAT-6 responses and, as hypothesized here, an incipient disease cut-off value that predicts subsequent development of progressive disease **(c,f)**.

severity of the HIV infection, they might have reduced ability to produce IFN-γ and it is therefore possible that another cut-off value should be applied for this group of patients. Few data on this are available, although what has been done indicates that IGRAs retain their utility unless CD4-positive T-cell numbers are greatly decreased [61].

Further studies have recently been launched to address these issues (Table 1). Although the study populations are different, the basic methodology is similar: screening of at-risk, but healthy, populations for their level of responsiveness to ESAT-6 and CFP-10 or the TST (after excluding active TB) and then following their clinical status over time, so that those who develop clinical disease can be identified. In all cases, TB is defined by a combination of sputum culture and/or smear, X-ray and clinical examination. Because TB can be self-limiting, active follow-up is planned for these studies to find those who develop active TB, without becoming so seriously ill that they seek hospitalization, otherwise the true disease rate is likely to be underestimated [51]. Although the studies have a basic common approach, they are also designed to answer slightly different questions.

The first five studies listed in Table 1 are all contact studies designed to estimate positive predictive values. By looking at close contacts of sputum-positive TB cases, who are at high risk of developing disease, the ratio of individuals expected to develop TB increases markedly, enabling accurate estimation of the positive predictive value of IGRA. The third study listed in Table 1 differs from the other contact studies in several important points. It focuses on immigrants to the Netherlands with prior TB exposure, which is a group with a traditionally high TB incidence (www.iphcr.res.in/html/RESEARCH-infectious.htm). However, because they are now resident in a region with very low TB incidence, acute re-infection can be ruled out as a significant factor. To the best of our knowledge, this is

Box 3. Outstanding questions

There are data indicating that a high response to ESAT-6 and CFP-10 reflects a serious infection. However, what constitutes a 'high' response is, to some extent, context-dependant and might differ from TB endemic to TB non-endemic regions and from HIV-uninfected to HIV-infected individuals. Furthermore, recent infection, long-term latent infection contained by host immunity and incipient disease are not characterized by identical immune responses.

Future studies therefore need to address the following issues:

- Can an incipient disease cut-off value for IGRAs be established so that a single measurement of the IFN-γ response to ESAT-6 and CFP-10 can simplify the clinician decision to treat an *M. tuberculosis*-infected but asymptomatic individual? Or alternatively, is it only on the basis of conversion (i.e. increasing levels of IFN-γ responses in two consecutive readings) that a decision can be made?
- Does the specificity and/or cytokine profile of the immune response to the pathogen change over time to include latency antigens? Can responses that are characteristic of the different stages of infection be identified?

Table 1. Summary of human studies examining the correlation between levels of ESAT-6- and CFP-10-induced IFN-γ response and subsequent progress to disease

Aim	Study location (principal investigator)	Readout	Refs	Study description	Number of participants	Start–end date
Positive predictive value	Hossana and Butajira, Ethiopia (T Mark Doherty)	TST, in house ESAT-6–CFP-10 ELISA[a]	www.iphcr.res.in/html/RESEARCH-infectious.htm	A prospective household contact study of asymptomatic contacts from households with an active TB case, plus community controls with no known TB contact, recruited locally. Two-year active follow-up at yearly intervals. Households identified through local TB clinics.	500	2003–2006
Positive predictive value	Istanbul, Turkey (Ajit Lalvani)	TST, T-SPOT.TB	[63]	A prospective household contact study of contacts from households with an active TB case. Two-year active follow-up. Households identified through local TB clinics.	979	2004–2008
Positive predictive value	The Netherlands (Martin Borgdorff)	TST, QFT, T-SPOT.TB	www.iphcr.res.in/html/RESEARCH-infectious.htm	A prospective contact study of asymptomatic immigrant TB contacts, with one-year active follow-up. Participants identified through municipal health services.	800	2005–2008
Positive predictive value	Zambia and South Africa (ZAMSTAR) (Peter Godfrey-Faussett, Helen Ayles and Nulda Beyers)	TST, QFT in tube	www.tbhiv-create.org/ZAMSTAR.htlm	A prospective household contact study in which adult and child contacts of newly diagnosed TB patients will be enrolled and followed for the development of TB over a three-year period.	8000	2007–2010
Positive predictive value	Palamaner Taluk, India (Mario Vaz)	TST, QFT, ESAT-6-based skin test	www.iphcr.res.in/html/RESEARCH-infectious.htm	A prospective household contact study of asymptomatic contacts from households with an active TB case, with two-year active follow-up at six monthly intervals. Households identified through local TB clinics.	400	2007–2010
Negative predictive value	Worcester, South Africa (William Hanekom)	TST, QFT	www.satvi.uct.ac.za/research.htm	A prospective cohort study of adolescents attending local high schools, with two-year follow-up. Cohort split equally between active (three monthly visits) and passive (entry and closeout visits only) arms for follow-up.	8000	2005–2008
Negative predictive value	Palamaner Taluk, India (Mario Vaz)	TST, QFT, ESAT-6-based skin test	www.iphcr.res.in/html/RESEARCH-infectious.htm	A prospective cohort study of adolescents attending local high schools, with two-year active follow-up. Cohort split equally between active (three monthly visits) and passive (entry and closeout visits only) arms for follow-up.	7500	2007–2010
Negative predictive value	Palamaner Taluk, India (Mario Vaz)	TST, QFT, ESAT-6-based skin test	www.iphcr.res.in/html/RESEARCH-infectious.htm	A prospective cohort study of all newborns recruited through the pregnancy registration system and followed actively by household visits and TB clinic surveillance for two years after birth.	4800	2007–2010

[a]ELISA, enzyme-linked immunosorbent assay.

the only study that was designed to compare directly the predictive value of the two IGRA assays commercially available. The final study in this group includes a novel skin test in which ESAT-6 has replaced PPD, in a way analogous to that used for the *in vitro* tests (www.iphcr.res.in/html/RESEARCH-infectious.htm).

The contact studies will be supplemented by larger cohort studies to determine negative predictive values (www.satvi.uct.ac.za/research.htm and www.iphcr.res.in/html/RESEARCH-infectious.htm). Study six and seven listed in Table 1 will enroll healthy 12–18 year-olds, thus covering the age range at which cases of pulmonary TB start to increase markedly. On the basis of earlier data, between 50 and 100 cases of TB are expected to develop during the course of these two studies, and this low number of TB cases should enable a precise definition of the negative predictive value of these tests. The adult studies will be supplemented by a study in infants in India (www.iphcr.res.in/html/RESEARCH-infectious.htm). All neonates born during the study period will be enrolled via local birth registers and, if they are found to have an identified TB contact or TB-like symptoms, they will receive a full clinical examination including immunodiagnosis. This is a group with high risk for both infection and serious disease, but one in which disease is often hard to diagnose and for which identifying technologies that could speed up diagnosis and identify incipient disease could be expected to save many lives.

These studies will all monitor the value of the test, not merely report a positive or negative result, and are pow-

ered so that they should produce sufficient data within the next few years to establish the efficacy of IGRAs in predicting incipient TB.

A prognostic test for incipient disease – even if it were only moderately accurate – would change current practice markedly and enable the prioritization of treatment to those at highest risk of developing contagious disease. This would enable the best use of scarce resources and, by treating latently infected individuals before they infect others, offer the possibility of breaking the cycle of transmission. Apart from a new vaccine that is active against latent TB, such an approach offers the best hope of achieving the final goal of controlling this longstanding global health emergency.

Concluding remarks

The development of highly specific IGRAs has enabled the accurate identification of TB infection, but it has not been possible so far to translate this into a better – or earlier – identification of contagious disease. A prognostic marker that enables targeted treatment of populations in high endemic regions that are in the process of developing contagious TB would greatly contribute to the control of this global epidemic. We hypothesize that, instead of using IGRAs only in a binary mode (infected and not infected) based on a cut-off value, the magnitude or conversion of an IGRA response might enable the identification of individuals who, although still asymptomatic, are in the process of developing active TB.

There are still several unresolved issues to address before the hypothesis discussed can have any effect on clinical practice (Box 3). However, these issues involve interpretation of results from existing technologies, not the invention of new tools. Although the observation that IFN-γ produced in response to specific antigens increases with increasing bacterial load seems simple enough to apply, ultimately the utility of this approach can be determined only by testing the hypothesis in large-scale cohort studies. This is the next challenge.

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References

1 WHO Report (2005) Global tuberculosis control – surveillance, planning, financing. World Health Organization (WHO/HTM/TB/2005.349)
2 Pai, M. *et al.* (2004) Interferon-γ assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect. Dis.* 4, 761–776
3 Lalvani, A. *et al.* (2001) Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. *Lancet* 357, 2017–2021
4 Brock, I. *et al.* (2004) Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. *Am. J. Respir. Crit. Care Med.* 170, 65–69
5 Mazurek, G.H. *et al.* (2005) Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recomm. Rep.* 54, 49–55
6 Kaufmann, S.H. (2001) How can immunology contribute to the control of tuberculosis? *Nat. Rev. Immunol.* 1, 20–30
7 Russell, D.G. (2001) *Mycobacterium tuberculosis*: here today, and here tomorrow. *Nat. Rev. Mol. Cell Biol.* 2, 569–577
8 Sherman, D.R. *et al.* (2001) Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding α-crystallin. *Proc. Natl. Acad. Sci. U.S.A.* 98, 7534–7539
9 Andersen, P. *et al.* (1991) T-cell proliferative response to antigens secreted by *Mycobacterium tuberculosis*. *Infect. Immun.* 59, 1558–1563
10 Leyten, E.M. *et al.* (2006) Human T-cell responses to 25 novel antigens encoded by genes of the dormancy regulon of *Mycobacterium tuberculosis*. *Microbes Infect.* 8, 2052–2060
11 Demissie, A. *et al.* (2006) Recognition of stage-specific mycobacterial antigens differentiates between acute and latent infections with *Mycobacterium tuberculosis*. *Clin. Vaccine. Immunol.* 13, 179–186
12 Wilkins, E.G. and Ivanyi, J. (1990) Potential value of serology for diagnosis of extrapulmonary tuberculosis. *Lancet* 336, 641–644
13 Weldingh, K. *et al.* (2005) Assessing the serodiagnostic potential of 35 *Mycobacterium tuberculosis* proteins and identification of four novel serological antigens. *J. Clin. Microbiol.* 43, 57–65
14 Wallis, R.S. *et al.* (1998) Induction of the antigen 85 complex of *Mycobacterium tuberculosis* in sputum: a determinant of outcome in pulmonary tuberculosis treatment. *J. Infect. Dis.* 178, 1115–1121
15 Choudhry, V. and Saxena, R.K. (2002) Detection of *Mycobacterium tuberculosis* antigens in urinary proteins of tuberculosis patients. *Eur. J. Clin. Microbiol. Infect. Dis.* 21, 1–5
16 Edwards, P.Q. and Edwads, L.B. (1960) Story of the tuberculin test from an epidemiologic viewpoint. *Am. Rev. Respir. Dis*. 81(1)Pt 2, 1–47
17 Judson, F.N. and Feldman, R.A. (1974) Mycobacterial skin tests in humans 12 years after infection with *Mycobacterium marinum*. *Am. Rev. Respir. Dis.* 109, 544–547
18 Mori, T. *et al.* (2004) Specific detection of tuberculosis infection: an interferon-γ-based assay using new antigens. *Am. J. Respir. Crit. Care Med.* 170, 59–64
19 Kang, Y.A. *et al.* (2005) Discrepancy between the tuberculin skin test and the whole-blood interferon γ assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. *J. Am. Med. Assoc.* 293, 2756–2761
20 Hill, P.C. *et al.* (2006) Comparison of enzyme-linked immunospot assay and tuberculin skin test in healthy children exposed to *Mycobacterium tuberculosis*. *Pediatrics* 117, 1542–1548
21 Jasmer, R.M. *et al.* (2002) Clinical practice. Latent tuberculosis infection. *N. Engl. J. Med.* 347, 1860–1866
22 Berkel, G.M. *et al.* (2005) Tuberculin skin test: estimation of positive and negative predictive values from routine data. *Int. J. Tuberc. Lung Dis.* 9, 310–316
23 Jeyakumar, D. (1999) Tuberculin reactivity and subsequent development of tuberculosis in a cohort of student nurses. *Med. J. Malaysia* 54, 492–495
24 Leung, C.C. *et al.* (2006) Risk of active tuberculosis among schoolchildren in Hong Kong. *Arch. Pediatr. Adolesc. Med.* 160, 247–251
25 Harboe, M. *et al.* (1996) Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis* BCG. *Infect. Immun.* 64, 16–22
26 Behr, M.A. *et al.* (1999) Comparative genomics of BCG vaccines by whole-genome DNA microarray. *Science* 284, 1520–1523
27 Andersen, P. *et al.* (2000) Specific immune-based diagnosis of tuberculosis. *Lancet* 356, 1099–1104
28 Andersen, P. *et al.* (1995) Recall of long-lived immunity to *Mycobacterium tuberculosis* infection in mice. *J. Immunol.* 154, 3359–3372
29 Shi, L. *et al.* (2004) Effect of growth state on transcription levels of genes encoding major secreted antigens of *Mycobacterium tuberculosis* in the mouse lung. *Infect. Immun.* 72, 2420–2424
30 Brodin, P. *et al.* (2006) Dissection of ESAT-6 system 1 of *Mycobacterium tuberculosis* and impact on immunogenicity and virulence. *Infect. Immun.* 74, 88–98
31 Pai, M. *et al.* (2006) New tools and emerging technologies for the diagnosis of tuberculosis: part I. Latent tuberculosis. *Expert Rev. Mol. Diagn.* 6, 413–422
32 Chapman, A.L. *et al.* (2002) Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of *Mycobacterium tuberculosis*-specific T cells. *AIDS* 16, 2285–2293
33 Taggart, E.W. *et al.* (2004) Evaluation of an *in vitro* assay for γ interferon production in response to *Mycobacterium tuberculosis* infections. *Clin. Diagn. Lab. Immunol.* 11, 1089–1093

34 Ferrara, G. *et al.* (2006) Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. *Lancet* 367, 1328–1334
35 Diel, R. *et al.* (2006) Tuberculosis contact investigation with a new, specific blood test in a low-incidence population containing a high proportion of BCG-vaccinated persons. *Respir. Res.* 7, 77
36 Shams, H. *et al.* (2005) Enzyme-linked immunospot and tuberculin skin testing to detect latent tuberculosis infection. *Am. J. Respir. Crit. Care Med.* 172, 1161–1168
37 Ewer, K. *et al.* (2003) Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet* 361, 1168–1173
38 Pai, M. *et al.* (2005) *Mycobacterium tuberculosis* infection in health care workers in rural India: comparison of a whole-blood interferon γ assay with tuberculin skin testing. *J. Am. Med. Assoc.* 293, 2746–2755
39 Arend, S.M. *et al.* (2006) Comparison of two interferon-γ assays and tuberculin skin test for tracing TB contacts. *Am. J. Respir. Crit. Care Med.* 175, 618–627
40 Diel, R. *et al.* (2006) Cost-optimisation of screening for latent tuberculosis in close contacts. *Eur. Respir. J.* 28, 35–44
41 Harada, N. *et al.* (2004) Basic characteristics of a novel diagnostic method (QuantiFERON TB-2G) for latent tubercolosis infection with the use of *Mycobacterium tubercolosis*-specific antigens, ESAT-6 and CFP-10. *Kekkaku* 79, 725–735
42 Harada, N. *et al.* (2006) Screening for tuberculosis infection using whole-blood interferon-γ and Mantoux testing among Japanese healthcare workers. *Infect. Control Hosp. Epidemiol.* 27, 442–448
43 Winslow, G.M. *et al.* (2003) Persistence and turnover of antigen-specific CD4 T cells during chronic tuberculosis infection in the mouse. *J. Immunol.* 170, 2046–2052
44 Dietrich, J. *et al.* (2005) Exchanging ESAT6 with TB10.4 in an Ag85B fusion molecule-based tuberculosis subunit vaccine: efficient protection and ESAT6-based sensitive monitoring of vaccine efficacy. *J. Immunol.* 174, 6332–6339
45 Buddle, B.M. *et al.* (1999) Differentiation between *Mycobacterium bovis* BCG-vaccinated and *M. bovis*-infected cattle by using recombinant mycobacterial antigens. *Clin. Diagn. Lab. Immunol.* 6, 1–5
46 Vordermeier, H.M. *et al.* (2002) Correlation of ESAT-6-specific γ interferon production with pathology in cattle following *Mycobacterium bovis* BCG vaccination against experimental bovine tuberculosis. *Infect. Immun.* 70, 3026–3032
47 Skinner, M.A. *et al.* (2003) A DNA prime–*Mycobacterium bovis* BCG boost vaccination strategy for cattle induces protection against bovine tuberculosis. *Infect. Immun.* 71, 4901–4907
48 Lyashchenko, K. *et al.* (2004) Association of tuberculin-boosted antibody responses with pathology and cell-mediated immunity in cattle vaccinated with *Mycobacterium bovis* BCG and infected with *M. bovis*. *Infect. Immun.* 72, 2462–2467
49 Langermans, J.A. *et al.* (2005) Protection of macaques against *Mycobacterium tuberculosis* infection by a subunit vaccine based on a fusion protein of antigen 85B and ESAT-6. *Vaccine* 23, 2740–2750
50 Ravn, P. *et al.* (1999) Human T cell responses to the ESAT-6 antigen from *Mycobacterium tuberculosis*. *J. Infect. Dis.* 179, 637–645
51 Doherty, T.M. *et al.* (2002) Immune responses to the *Mycobacterium tuberculosis*-specific antigen ESAT-6 signal subclinical infection among contacts of tuberculosis patients. *J. Clin. Microbiol.* 40, 704–706
52 Demissie, A. *et al.* (2004) Healthy individuals that control a latent infection with *Mycobacterium tuberculosis* express high levels of Th1 cytokines and the IL-4 antagonist IL-4δ2. *J. Immunol.* 172, 6938–6943
53 Hill, P.C. *et al.* (2005) Quantitative T cell assay reflects infectious load of *Mycobacterium tuberculosis* in an endemic case contact model. *Clin. Infect. Dis.* 40, 273–278
54 Pai, M. *et al.* (2006) Serial testing of health care workers for tuberculosis using interferon-γ assay. *Am. J. Respir. Crit. Care Med.* 174, 349–355
55 Corbett, E.L. *et al.* (2006) Tuberculosis infection in African nursing students: tuberculin skin test compared to ELISPOT conversion rates. *Int. J. Tuberc. Lung Dis.* 10, 231
56 Whalen, C.C. *et al.* (2006) Immune correlates of acute *Mycobacterium tuberculosis* infection in household contacts in Kampala, Uganda. *Am. J. Trop. Med. Hyg.* 75, 55–61
57 Menzies, D. (1999) Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. *Am. J. Respir. Crit. Care Med.* 159, 15–21
58 Pathan, A.A. *et al.* (2001) Direct *ex vivo* analysis of antigen-specific IFN-γ-secreting CD4 T cells in *Mycobacterium tuberculosis*-infected individuals: associations with clinical disease state and effect of treatment. *J. Immunol.* 167, 5217–5225
59 Carrara, S. *et al.* (2004) Use of a T cell-based assay for monitoring efficacy of antituberculosis therapy. *Clin. Infect. Dis.* 38, 754–756
60 Aiken, A.M. *et al.* (2006) Reversion of the ELISPOT test after treatment in Gambian tuberculosis cases. *BMC Infect. Dis.* 6, 66
61 Dheda, K. *et al.* (2005) Performance of a T-cell-based diagnostic test for tuberculosis infection in HIV-infected individuals is independent of CD4 cell count. *AIDS* 19, 2038–2041
62 Doherty, T.M. and Andersen, P. (2005) Vaccines for tuberculosis: novel concepts and recent progress. *Clin. Microbiol. Rev.* 18, 687–702
63 Soysal, A. *et al.* (2005) Effect of BCG vaccination on risk of *Mycobacterium tuberculosis* infection in children with household tuberculosis contact: a prospective community-based study. *Lancet* 366, 1443–1451