REVIEW

Arch. Immunol. Ther. Exp., 2009, **57**, 425–433
PL ISSN 0004-069X
DOI 10.1007/s00005-009-0050-9

Exploring the immune response against *Mycobacterium tuberculosis* for a better diagnosis of the infection

Giovanni Ferrara[1], Monica Losi[2, 3], Leonardo M. Fabbri[3], Giovanni B. Migliori[4], Luca Richeldi[2, 3] and Lucio Casali[1]

[1] Section of Respiratory Disease, Department of Internal Medicine, St. Maria Hospital, University of Perugia, Terni, Italy
[2] Center for Rare Lung Diseases, University of Modena and Reggio Emilia, Modena, Italy
[3] Sections of Respiratory Diseases, Department of Oncology and Hematology, University of Modena and Reggio Emilia, Modena, Italy
[4] WHO Collaborating Centre for TB and Lung Diseases, Fondazione S. Maugeri, Care and Research Institute, Tradate, Italy

Received: 2009.02.11, **Accepted:** 2009.04.29, **Published online:** 2009.10.29

Abstract

Tuberculosis (TB) still represents a monumental problem, with more than two million deaths every year worldwide. The current diagnostics for TB offer sub-optimal accuracy both for the active and the latent form of infection and are often based on technologies unaffordable in low-income settings. The tuberculin skin test was the first diagnostic based on an acquired immune response towards *Mycobacterium tuberculosis* (MTB). Advances in molecular and cellular biology and the elucidation of the mechanisms governing the relation between MTB and the human immune system form the basis for new and more accurate assays, potentially able to fill the gaps and limits of classical diagnostics. However, the process of validating new tests is still complex and hampered by specific questions regarding TB immunology and natural history. We present here a summary of the current approaches to validate new diagnostics based on the detection of immunological biomarkers of TB infection.

Key words: tuberculosis, acquired immune response, latent tuberculosis infection, diagnosis, new tools.

Corresponding author: Giovanni Ferrara, MD, Ph.D., Section of Respiratory Disease, Dept. of Internal Medicine, St. Maria Hospital, University of Perugia, Via T. Di Joannuccio 1, 05100 Terni, Italy, tel.: +39 744-205686, fax: +39 744-404217, e-mail: gferrara@unipg.it

INTRODUCTION

Tuberculosis (TB) still represents a monumental problem in the world, with almost 2 million deaths every year (World Health Organization 2008). The lack of fast, easy, accurate, and affordable diagnostics is one of the major factors limiting the fight against this infectious disease.

Robert Koch was the first to understand that smear microscopy could detect less than 50% of active TB cases, but still today, after more than a century, this is the only diagnostic available and affordable worldwide, especially in developing countries, where the highest rates of disease are reported (Perkins and Cunningham 2007). Koch's work was fully oriented not only towards improving TB diagnosis, but also to understanding the dynamics of the disease, based on the concept that *Mycobacterium tuberculosis* (MTB), once in the human body, is able to trigger a sequence of events leading to specific pathological lesions. In doing so, he postulated the basis of modern immunology, understanding that granulomas are not a self-destroying event due to a process of decay of human material, as believed at the end of the 1800s, but instead an organized, distinct, and specific cellular response to MTB infection (Kaufmann and Winau 2005). This concept was further strengthened by the discovery of the "Koch phenomenon", the skin reaction observed with the inoculation of tuberculin in patients with active TB and in their contacts. Again, the concept of a reaction of the body to an external pathogen and the possibility to use it to treat TB were clear in Koch's mind (Rook and Stanford 1996).

Unfortunately, this attempt failed, but left a powerful tool for the diagnosis of infection, the tuberculin skin test (TST). With the TST, the first biomarker of an infectious disease was discovered. Despite the diffusion and the impact of this test in 100 years of clinical practice, and despite the fact that its immunological basis is beyond doubt, the fine mechanisms underlying the events leading to the Koch phenomenon are still not fully unmasked. Still, Koch's main intuition represents a precious legacy: once the human body (in particular, the immune system) encounters MTB, something changes forever and influences the fate, the natural history, and the possibility to overcome the pathogen. This concept is the rationale for the discovery and application of new biomarkers.

Thanks to the unraveling of the sequence of the MTB genome (Cole et al. 1998), which offered the possibility to select specific recombinant antigens, and to the continuous advances in cellular and molecular biology, today it is possible to use new technologies to explore the relation between MTB and the immune system. However, despite the many methods developed in the last 20 years with proof of concept to develop new TB diagnostics, only a few attempts managed to go through all the clinical phases of a new test (Table 1), to appear on the market, and to show a real advantage in terms of outcome in the subjects undergoing the test (European Medicines Agency 2008; Sackett and Haynes 2002). Major obstacles of this process are the nature of TB infection itself (years may be necessary to have data on incident cases once the infection is acquired, and ethical problems are evident in case a diagnostic test and a treatment are already available) and the lack of a gold standard for latent TB infection (LTBI), forcing the interpretation of results of new assays on surrogate gold standards, such as the intensity of exposure and active TB, with all the limits these models imply (Lalvani 2007). Furthermore, due to the nature of LTBI in humans, animal models are not available and suitable for testing new tools in infection models prepared in the laboratory.

In this review, we try to summarize the current attempts to use the specific immune response triggered by the MTB as a powerful marker to develop more accurate and long-awaited diagnostic tools for TB infection.

THE IMMUNE RESPONSE IN TB

The main entrance of MTB into the human body is the respiratory system, due to the spread of the bacilli in the air through Pflugge droplets from a contagious TB case (Rieder 1999), and macrophages are the first cells to encounter the bacillus. It is now clear that in the early phases of infection, MTB is able to hijack the intracellular mechanisms of pathogen killing in macrophages, inducing cytokines and chemokines related to tolerance (Ferrara et al. 2008) and inhibiting the formation of phagolysosome (Kusner 2005; Vergne et al. 2005). This creates an ideal environment for MTB replication and growth. Dendritic cells are resistant to the infection and are postulated to migrate into the secondary lymphoid tissues, where they present MTB antigens to naïve T cells on MHC-I, MHC-II, and CD1 (Salgame 2005). At this point, specific CD4 and CD8 T cells and unconventional γδ and CD1-restricted T cells are primed and activated and, subsequently, recruited to the site of infection (Flynn 2004). Once this process starts, the immune system acquires specific effective and, subsequently, memory cells that will be present until the death of the infected subject. T helper 1 (Th1) cells play a major role through the production of interferon (IFN)-γ and the induction of apoptotic mechanisms in the cells hosting MTB, leading to bacterial death (Flynn 2004). The T cells' repertoire (i.e. their ability to recognize MTB epitopes presented on the surface of infected cells) will form the main response leading to granuloma formation and infection containment, influencing susceptibility to or protection from the disease (Boom et al. 2003). In this sense, susceptibility to MTB is also driven by the HLA allelic variants of the infected subject, being well known that certain alleles are strongly associated with the risk of progression to active disease. A recent computerized model suggests that the HLA-DR alleles HLA--DRB1*0801, *0802, *1401, *1501, and *1502 (associated with TB susceptibility) are able to recognize and present a significantly lower number of MTB epitopes than the HLA-DR alleles HLA-DRB1*0301, *0701, *1101,*1102, *1301, and *1302 (associated instead with protection) (Contini et al. 2008). Therefore, the number (and probably the type) of MTB epitopes recognized by the immune system and the affinity of HLA class I and II could be one of the most important determinants of susceptibility to TB, concurring with other risk factors, such as HIV co-infection and other medical conditions, to the fate of the infection. Similarly, the ability to recognize MTB epitopes also influences the priming of B cells and the production of specific immunoglobulins (Ig). Although the role of these proteins in controlling the infection is still debated, they offer another potential marker of infection (Flynn 2004).

This chain of events leading to a specific acquired cellular response seems to contain, more than to eradicate, the TB infection. It is well known, in fact, that once infected with MTB, the host has a definite risk of progression to active disease that correlates with the time of the infection, the first two years being crucial (American Thoracic Society 2000; Rieder 1999), and with medical conditions impairing the Th1 response, first of all HIV co-infection and treatment with immunosuppressive drugs (Horsburgh 2004). The need to diagnose LTBI and predict the risk of progression to active disease arises from these considerations. The main diagnostics based on the specific immune response to MTB and their principles will be presented in the next paragraphs (Fig. 1).

Table 1. Summary of the phases of trials to validate new diagnostics for tuberculosis (TB) infection based on immunological biomarkers

Study phase	Skin test	T cell-based assays			Antibody-detection assays	
Phase I	rdESAT-6 skin test (+)					Assays based on libraries of epitopes (+)
Phase II		Flow cytometry (blood and sputum) (+) IGRAs performed on pleural, BAL, and CNS fluids (+)	New markers (IP-10, IL-10, MCP-2) or new antigens (+)	Single antigen detection tests (–)	Combined antigens detection tests (–)	
Phase III		IGRAs (on blood cells for TB infection) (+) (–)*				
Phase IV	TST					

Phase I: the marker is expressed in a statistically significant manner in patients with TB infection compared with infection-free subjects; Phase II: the test has a good accuracy, distinguishing patients with already certain, diagnosed TB infection from infection-free subject; Phase III: the test has a good accuracy in distinguishing patients with TB infection from those without among subjects at high risk of infection; Phase IV: the use of test improves the outcome of patients who undergo the test compared with those who do not or who undergo another test for the same condition. (+): the results of studies in the phase corresponding to the left column suggest the possibility to pass to the next phase based on good test accuracy; (–): results of studies in the phase corresponding to the left column do not support further studies; TST: tuberculin skin test; rdESAT-6: recombinant dimer early secretory antigen target 6; IGRA: IFN-γ-release assays; BAL: bronchoalveolar lavage; CNS: central nervous system; IP-10: IFN-γ-inducible protein 10; MCP-2: monocyte chemotactic protein 2; *Phase III: studies on IGRAs showed good predictive values for latent tuberculosis infection (+), but suboptimal values when assays are used as rule-out test for active TB (–).

THE TST AND ITS EVOLUTION

Tuberculin was the first diagnostic test for LTBI and Koch himself understood the possibility of diagnosing TB in smear-negative subject (and infection in healthy contacts) by inoculating this cocktail of about 200 MTB antigens in the skin (Kaufmann and Winau 2005). Today, the potentiality, limits (no distinction between LTBI and active TB, LTBI and vaccination/non-tuberculous mycobacteria (NTM)), and the predictive values of the TST are well known. The test is routinely used to diagnose LTBI in all groups at increased risk of reactivation. A complete summary of the literature about the TST was published by the Centers for Disease Control and Prevention together with the American Thoracic Society in 2000 and it comprises recommendations for its use and interpretation (American Thoracic Society 2000). Despite the wide body of evidence on the use of the TST in clinical practice (it is the only test with available phase IV studies, Table 1), the cellular and molecular mechanisms occurring in the skin of latently infected subjects after tuberculin inoculation are still mostly unknown. Furthermore, what we know today about the immune reaction triggered by tuberculin in the skin does not correlate with induration size, the clinical parameter measured to deem a TST positive or negative. Classically interpreted as a delayed type hypersensitivity reaction to tuberculin (Vukmanovic-Stejic et al. 2006), a very elegant study performed on skin biopsies at predetermined time points showed recently that CD4 T cells are strongly involved, but the skin infiltration and the local proliferation of these cells were maximal only 7 days after the inoculation, well after the time limit of 72 h to "read" the TST and to interpret its result in millimeters of induration (Reed et al. 2004). Most of the cells harvested from biopsies are lymphocytes and show a $CD45RA^{-}(CD45RO^{+})$ primed/memory phenotype, supporting the idea that tuberculin induces a CD4 memory T-cell immune response (Vukmanovic-Stejic et al. 2008), but what happens during the first 3 days, and what thus empowers the test as a powerful diagnostic for LTBI, is still mostly unknown.

Although our knowledge of the fine immunological mechanisms leading to skin induration is limited, TST is today one of the most used diagnostic tests in clinical practice and the only test for TB infection with a strong

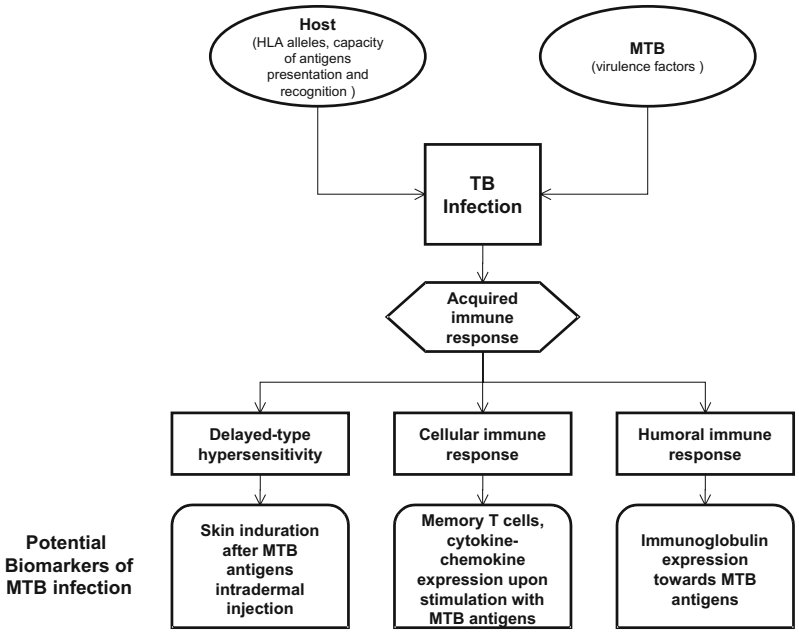

Fig. 1. Flow-chart summarizing the passages from the encounter of the host and *Mycobacterium tuberculosis* (MTB) in the pathogenesis of tuberculosis (TB) infection and the development of an acquired immune response towards the bacterium. The different ways MTB epitopes are recognized by the immune system and the immunological reactions MTB antigens can trigger offer the possibility to test several biomarkers for TB infection.

body of evidence of the regarding its accuracy and predictive values in most risk groups. Advantages of the TST are that it is simple to perform, there is no need of a laboratory, and it is inexpensive and can therefore also be used in resource-limited settings. The sensitivity is very high in immunocompetent subjects, but it falls dramatically in the presence of an impaired immune response, i.e. HIV co-infection with low CD4 counts or ongoing immunosuppressive treatment. The specificity of the TST is reduced by vaccination with the Bacillus Calmette-Guérin (BCG) and by the infection with NTM due to the fact that these bacteria express most of the antigens included in the purified protein derivative (PPD) of the TST, causing false-positive results in patients who are not latently infected by MTB (Huebner et al. 1993; Menzies 1999). For these reasons, the TST is considered positive with different cut-off values (≥5 mm, ≥10 mm, ≥15 mm), which try to optimize sensitivity and specificity according to the confounding factors of the population tested (American Thoracic Society 2000).

An attempt to improve the skin test was recently made using a recombinant dimer of early secretory antigen target 6 (rdESAT-6), a protein encoded by the region of difference 1 (RD1) of the MTB chromosome. This region, and thus the proteins and their epitopes, is not present on BCG and on most NTMs, making it a perfect candidate for a more specific skin test. In fact, inoculation of rdESAT-6 in the skin of subjects with LTBI or previous TB was proven to induce local responses similarly to TST, but no skin reaction was observed in BCG-vaccinated individuals (Aggerbeck and Madsen 2006). A phase I study comparing rdEAST-6 and TST is available showing its feasibility, and providing some data on safety and the proof of principle for further investigations in humans (Arend et al. 2008).

An attempt to develop a transdermal patch delivering a recombinant antigen (MTB-64) was also done in the past, but with few and unencouraging results (Nakamura et al. 2001).

T CELL-BASED ASSAYS

T cells are essential in controlling TB infection in the human body and their priming and activation are the main events leading to an acquired immune response against MTB. Similarly to the skin reaction induced by tuberculin, the detection of specific T cells able to recognize and respond to MTB epitopes through cytokine expression can occur only if the immune system of the cell donor already encountered the bacillus and developed a specific Th1 response. Therefore, the possibility to detect specific T cells implies the possibility to detect TB infection.

Two methods were introduced to identify T cells, or rather their specific response, a decade ago, and today they are the technological platforms of two commercial diagnostics used worldwide. The first paper about the use of the enzyme-linked ImmunoSpot (ELISpot) assay was published in 1998 and showed that it was possible to detect T cells producing IFN-γ in response to ESAT-6 peptides. In this assay, the capture of IFN-γ at the bottom of the well was revealed with a colorimetric reaction showing the footprints of every cell responding to the specific stimulation (Lalvani et al. 1998). Thus it was possible to count how many T cells specific for the MTB antigens were present in the peripheral blood mononuclear cells (PBMCs) of a subject with active TB. The other format used to detect the immune response in a blood sample of 1 ml to MTB antigens was the enzyme-linked immunosorbent assay (ELISA), which

gave the opportunity to dose the quantity of IFN-γ produced upon stimulation with PPD or specific peptides. The amount of IFN-γ produced by subjects with LTBI or active TB was significantly different from the response in uninfected individuals, showing a potential marker for infection potentially detectable in any laboratory (Demissie et al. 1999).

The first versions of these assays used PPD and/or home-made peptides. It was soon shown that the best antigens to evoke IFN-γ production from the T cells of infected subjects were ESAT-6 and another protein called culture-protein filtrate 10 (CFP10), also expressed by the RD1 genes (Lalvani et al. 1998). The combination of the epitopes contained in these two proteins ensured the best sensitivity and specificity of the tests (today referred to as IFN-γ-release assays, or IGRAs), as shown by phase II studies. Active TB (Mori et al. 2004) or the intensity of exposure (Brock et al. 2004; Lalvani et al. 2001; Richeldi et al. 2004) to a contagious TB case were generally used as a surrogate gold standard of infection, and a recent meta-analysis of these studies showed that IGRAs are at least as sensitive and certainly more specific than the TST (Pai et al. 2008). The first longitudinal data showed good IGRA predictive values for TB reactivation in immunocompetent subjects, even superior to TST when BCG-vaccinated subjects are investigated (Bakir et al. 2008; Diel et al. 2008). The high sensitivity of the assays in phase II studies proposed the IGRAs as potential rule-out tests for active TB. Despite the enthusiasm of the first data, a multicenter prospective phase III study showed that the sensitivity of the blood tests can fall below 70% when they are used in the clinical practice in unselected populations, so today their use is not recommended for active TB (Mazurek et al. 2007).

The potentials of IGRAs in subjects with impaired immune response is still to be fully evaluated. Although the first phase II studies showed higher sensitivities of the IGRAs in patients with active TB and AIDS (Liebeschuetz et al. 2004), it is known that the type and the degree of immunosuppression affect the performance of the assays, influencing the rates of indeterminate and of discordant results (Dheda et al. 2005; Ferrara et al. 2006; Ferrara et al. 2005) among different risk groups (Richeldi et al. 2009). A potential advantage of the IGRAs over the TST is the inclusion in their format of a positive and a negative control, giving a certain degree of reliability to the test results. Again, the first longitudinal data obtained with a home-made RD1, ELISpot, suggest that in risk groups with possible immunosuppression, the predictive value of IGRAs can be reduced. In this setting, the sensitivity of the screening procedures could be improved by a combination of IGRAs with the TST results, helping to prevent a higher proportion of incident TB cases (Hill et al. 2008). The role of IGRAs in low-income settings is still to be evaluated, also considering their cost, and their use is currently recommended only in reference centers (Perkins and Cunningham 2007).

Other clinical applications for IGRAs suggested the possibility of monitoring the T-cell response over time and to explore somehow the dynamics of the immune response with a simple tool. Initial studies of commercial IGRAs as potential markers of adherence to treatment and outcome evaluation led to inconclusive results for both active and latent TB (Carrara et al. 2004; Chee et al. 2007; Dheda et al. 2007). A report from Japan suggests that the response in QuantiFERON©-TB Gold can vanish over time after the infection (Mori et al. 2007), helping to distinguish a recent from a remote infection, but the impact of this observation in clinical practice is far from be proven. The role of IGRAs in differentiating patients with latent and active TB has also been investigated. Although some studies suggest that the response in patients with active TB could be quantitatively stronger than in patients with LTBI, a great variability has also been recorded (Janssens et al. 2007).

A promising approach in the diagnosis of active TB forms is offered by the fact that T cells are selectively recruited and activated at the site of disease. The possibility to purify T cells from other bodily fluids and to test their response to MTB antigens was shown to have very high sensitivity and good specificity in the diagnosis of smear-negative pulmonary TB cases using cells in the BAL (Jafari et al. 2006), pleural fluids (Losi et al. 2007), and the central nervous system (Thomas et al. 2008). The value of this approach on unselected cohorts of patients is still to be evaluated.

Most of the studies are performed today with commercial versions of these assays, the T-SPOT.TB (Oxford Immunotech, Abingdon, UK) for the ELISpot and the QuantiFERON©-TB Gold in tube (Cellestis Ltd, Carnegie, Australia) for the ELISA platform. These assays are now used worldwide and their relatively simple technologies make them the most likely candidates to replace the TST in the near future, despite the lack of information on their effectiveness in TB control strategies in both Western and developing countries. The ELISA platform is definitely easier to use, requires instruments that are part of the equipment of most of the laboratories, and presents logistical advantages (samples can be shipped and reading repeated); this makes it an affordable test almost everywhere. The ELISpot platform requires more skills and dedicated instruments, even though the diffusion of the ELISpot-readers is growing, but it is more controlled and can also be performed on samples other than blood. In any case, these two assays will have a huge impact on TB control and prevention in the near future, and data from phase IV studies are awaited to show their potential advantages.

Different strategies are being investigated to overcome the limits shown by IGRAs. An approach to improve IGRAs sensitivity was recently proposed, boosting the IFN-γ response by adding interleukin (IL)-7 to the lymphocyte stimulation with MTB antigens (Feske et al. 2008). Another approach includes an increase in the time of incubation from 16 h to up to 5–7

days (Leyten et al. 2007). Phase I and II trials on different technological platforms potentially able to provide more informative and precise data were published. Among them, studies performed with flow cytometric assays to detect the T-cell response to MTB antigens seem to provide data not only on the cytokines produced, but also on the lymphocyte subsets (Leung et al. 2009). Data on the correlation of these tests' results and active TB are already available for BAL, sputum, and blood (Breen et al., 2008; Breen et al. 2007), showing good sensitivities. The richer body of information provided by flow cytometry could open new possibilities and solve important problems in the clinical evaluation of patients with TB infection (Morner 2006).

Data are also available on new markers of the T-cell response. IFN-γ-inducible protein-10, monocyte chemotactic protein-2, and IL-10 showed good accuracy for active TB in phase II studies (Ruhwald et al. 2008a; Ruhwald et al. 2008b; Ruhwald et al. 2009) and new antigens able to evoke the T-cell response have been proposed (Geluk et al. 2007). Proof of concept is also available on a new test based on the differential RNA expression induced by PBMC stimulation with ESAT-6. Based on preliminary results, it could be possible to differentiate active and latent TB based on different levels of expression detected with quantitative real-time PCR for the genes for IL-8, FOXP3, and IL-12β (Wu et al. 2007). Phase II studies are awaited. Furthermore, MHC class I and II tetramer technology offers a new way to detect the specific T cells responding to MTB antigens (Höhn et al. 2007).

BLOOD TESTS BASED ON SEROLOGICAL ANTIBODY DETECTION FOR MTB ANTIGENS

Antibody detection tests are used to diagnose several viral and bacterial diseases. During the progression to active TB, the priming and expansion of B lymphocyte subsets occur toward MTB antigens, causing a serological response with the production of IgG, IgM, and IgA (Flynn 2004). Although it is clear that serological immunity has a very limited role in controlling TB infection, it potentially offers the opportunity to identify specific, easy-to-detect, and inexpensive markers. Unfortunately, the great variability in antibody expression for most of the known MTB antigens has limited the development of reliable serological tests for TB (Abebe et al. 2007).

The presence of specific immunoglobulins in patients with active TB were tested by hemoagglutination assay already in 1898, but it was not until the last 30 years that the development of more reliable techniques, such as ELISA, and improvements in antigen purification allowed the use of antibody detection as a marker of active TB. The major problems were due to the poor specificity of the antigens used in the assays, often derived from culture filtrates or from the same PPD. Furthermore, the nature of these antigens, such as lipoprotein or glycolipids, which already have reduced immunogenic capacity, and the fact that they are often contained in other bacterias' cell walls, limited the development of assays with good sensitivity and specificity. The 38-kDa antigen was one of the first and most used antigens to detect specific antibodies in patients with active TB (Amicosante et al. 1995). Most of the assays were based on an ELISA platform, and some of them were also available on the market. Unfortunately, a great variability in sensitivity (ranging 40–89%) and specificity (44–100%) limited the implementation of these assays in clinical practice (Abebe et al. 2007). Several antigens have been evaluated in the same way (19-kDa lipoprotein, 16-kDa antigen, MTB81, antigen 60, to cite only a few), but without achieving convincing accuracy in phase II studies. Highly preserved amino-acid sequence motifs present in proline-glutamine and proline-proline-glutamine antigens and glycolipids such as lipoarabinomannan showed the same problems, and no improvement was made by investigating different Ig classes (IgM, IgA, and IgG, separately or together) or by using different technological platforms, such as Western blotting. Sequencing of the MTB DNA and the discovery of new potential antigens/epitopes open new possibilities in this field of research, but so far, recombinant antigens, such as the RD1-encoded ESAT-6 and CFP10, were not useful in improving the serological diagnosis of active TB.

Nevertheless, in low-income countries many serological assays are on the market due to the lack of regulatory authority (Steingart et al. 2007b). The WHO Special Program for Research and Training in Tropical Diseases commissioned two systematic reviews on the use of these assays to diagnose pulmonary and extrapulmonary TB. In the first review, methods were classified on the basis of the antigens used (recombinant proteins, native proteins, lipids, multi-antigen lipid-lipid, protein-protein, and protein-lipid). Suboptimal sensitivity and specificity for the diagnosis of pulmonary TB was reported after analyzing the single methods. Protein antigen-based tests and a test using multiple antigens showed higher sensitivities among smear-positive TB patients, while limited data were available on pulmonary smear-negative, pediatric, and HIV-co-infected TB cases (Steingart et al. 2009). Similar results were reported in a systematic review on the use of antibody detection tests for the diagnosis of extrapulmonary TB (Steingart et al. 2007a), so that these assays are not recommended in the diagnosis of any form of TB in the present formats.

Although the methods used so far have failed to provide an accurate diagnostic based on antibody detection, the perspective of having a fast and, likely, very easy test without the need for handling contagious samples (such as sputum) is still very attractive and potentially extremely important to fight TB, especially in resource-limited settings. The problem of the low sensitivity of single antigens could be overcome by new technologies, such as Luminex and proteomics, that could allow testing a library of antigens, improving sensitivity without losing specificity. The proof of concept of this approach is already available in animal models (Khan et al. 2008).

The other problem to solve is the great variability in specific antibody expression in different subjects. Again, the affinity of HLA-class I and II alleles for MTB antigens could be involved and could determine the pattern of circulating antibodies. Therefore, the capability of recognizing MTB antigens and of mounting a B-cell response is likely based on the genome of the infected subject. Preliminary data in humans obtained with proteomic chips seem to lend support to this hypothesis and could be the basis to develop multi-antigen tests able to recognize patterns of antibodies in different ethnic groups, improving the overall performance and accuracy of antibody detection-based diagnosis of TB (Gaseitsiwe et al. 2008; Hoff et al. 2007).

SUMMARY AND FUTURE PERSPECTIVES

The detection of biomarkers related to an acquired immune response against MTB offers powerful tools for diagnosing TB infection. The relation between MTB and the delayed type hypersensitivity reaction to tuberculin was used just a decade after the discovery of the pathogen, and still today the TST represents the main diagnostic for LTBI worldwide, being standardized, easy-to-perform, inexpensive, and provided with a good body of evidence about its predictive values in all TB risk groups. Its drawbacks regarding sensitivity (in immunocompromised patients) and specificity (in subjects with a BCG vaccination or NTM infection) are very well known and are an impetus for the development of more accurate diagnostics. The increased knowledge of MTB structure and biology, the possibility to synthesize recombinant purified antigens, and the better understanding of the mechanisms underlying the acquired immune response to the pathogen give the opportunity to test new biomarkers for TB infection (Jacobsen et al. 2008) potentially able to overcome the limits of the old tuberculin. Phase I studies showed the potential use of recombinant antigens instead of the PPD for more specific skin tests. The possibility to detect *in vitro* the T-cell response to recombinant antigens such as ESAT-6 and CFP10 led to the development of the only two commercial tests (QuantiFERON©-TB Gold and T-SPOT.TB) with good sensitivity and higher specificity than the TST and based on technologies affordable to most laboratories in Western countries. Their wide use in clinical practice, the availability of phase III studies, and the initial data on their predictive values make them the best candidates to substitute TST in the near future. Data are still missing on their accuracy and predictive values in immunocompromised patients, and thus combined use with the TST is now recommended by most international guidelines on the diagnosis of LTBI. Similarly to the TST, their use in the diagnosis of active TB forms is not recommended due to the reduced sensitivity reported in phase III studies on this application. Initial promising data are available on new T cell-based diagnostics based on the simultaneous detection of multiple cytokines or on different technological platforms such as flow cytometry.

The initial enthusiasm about antibody detection tests (potentially easy to perform, safer than biological materials such as sputum, and based on simple technologies and on samples that can be stored for a long time) was soon disappointed by the low sensitivity and the variability in immunoglobulin expression in different populations.

New technologies now offer very interesting perspectives. The possibility to explore the immune response against MTB with libraries of antigens/epitopes with microarrays and customized chips enables to select the best antigens and the best combination to develop highly sensitive and specific diagnostics. Understanding HLA class I and II alleles will also allow to select the best antigens for different ethnic groups around the world and to use different epitopes to evoke biomarkers for susceptibility to active disease or protection. The detection of specific gene expression in PBMCs stimulated with recombinant antigens hold interesting promises to differentiate remote and recent infection and active disease.

Finally, the possibility to detect multiple biomarkers (cytokines, chemokines, antibodies, and gene expression) simultaneously and to characterize the different memory/effector cell subsets (and their genomes) involved in the phases of infection will improve the diagnosis of TB in difficult risk groups, such as immunocompromised subjects, and will allow to distinguish all the infection phases and the disease's natural history in all ethnic groups. This will be even more important as the new diagnostics are mostly needed where the TB epidemic today accounts for 1.7 million deaths every year, i.e. Sub-Saharan Africa, South-East Asia, and China. Research efforts in this very important area must be dedicated to the development of tools to improve TB diagnosis in low-income and extreme poverty settings and adapting (or inventing) new technologies to operational needs. In this context, easy-to-perform, accurate, quick, and safe tools without the need of handling infectious materials could represent a milestone in the fight against TB. The political commitments of all the organizations working in this fight will be a crucial point in making the new tools affordable also in poorer settings (Perkins and Cunningham 2007).

REFERENCES

Abebe F, Holm-Hansen C, Wiker HG et al (2007) Progress in serodiagnosis of Mycobacterium tuberculosis infection. Scand J Immunol 66:176–191

Aggerbeck H, Madsen SM (2006) Safety of ESAT-6. Tuberculosis 86:363–373

American Thoracic Society (2000) Targeted tuberculin testing and treatment of latent tuberculosis infection. Am J Respir Crit Care Med 161(suppl):S221–247

Amicosante M, Barnini S, Corsini V et al (1995) Evaluation of

a novel tuberculosis complex-specific 34 kDa protein in the serological diagnosis of tuberculosis. Eur Respir J 8:2008–2014

Arend SM, Franken WP, Aggerbeck H (2008) Double-blind randomized Phase I study comparing rdESAT-6 to tuberculin as skin test reagent in the diagnosis of tuberculosis infection. Tuberculosis 88:249–261

Bakir M, Millington KA, Soysal A et al (2008) Prognostic value of a T-cell-based, interferon-gamma biomarker in children with tuberculosis contact. Ann Intern Med 149:777–787

Boom WH, Canaday DH, Fulton SA et al (2003) Human immunity to M. tuberculosis: T cell subsets and antigen processing. Tuberculosis 83:98–106

Breen RA, Barry SM, Smith CJ et al (2008) Clinical application of a rapid lung-orientated immunoassay in individuals with possible tuberculosis. Thorax 63:67–71

Breen RA, Hardy GA, Perrin FM et al (2007) Rapid diagnosis of smear-negative tuberculosis using immunology and microbiology with induced sputum in HIV-infected and uninfected individuals. PLoS One 2:e1335

Brock I, Weldingh K, Lillebaek T 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

Carrara S, Vincenti D, Petrosillo N et al (2004) Use of a T cell--based assay for monitoring efficacy of antituberculosis therapy. Clin Infect Dis 38:754–756

Chee CB, KhinMar KW, Gan SH et al (2007) Latent tuberculosis infection treatment and T-cell responses to Mycobacterium tuberculosis-specific antigens. Am J Respir Crit Care Med 175:282–287

Cole ST, Brosch R, Parkhill J et al (1998) Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature 393:537–544

Contini S, Pallante M, Vejbaesya S et al (2008) A model of phenotypic susceptibility to tuberculosis: deficient in silico selection of Mycobacterium tuberculosis epitopes by HLA alleles. Sarcoidosis Vasc Diffuse Lung Dis 25:21–28

Demissie A, Ravn P, Olobo J et al (1999) T-cell recognition of Mycobacterium tuberculosis culture filtrate fractions in tuberculosis patients and their household contacts. Infect Immun 67:5967–5971

Dheda K, Lalvani A, Miller RF 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

Dheda K, Pooran A, Pai M et al (2007) Interpretation of Mycobacterium tuberculosis antigen-specific IFN-gamma release assays (T-SPOT.TB) and factors that may modulate test results. J Infect 55:169–173

Diel R, Loddenkemper R, Meywald-Walter K et al (2008) Predictive value of a whole blood IFN-gamma assay for the development of active tuberculosis disease after recent infection with Mycobacterium tuberculosis. Am J Respir Crit Care Med 177:1164–1170

European Medicines Agency (2008) Guideline on Clinical Evaluation of Diagnostic Agents. CPMP/EWP/1119/98/Rev 1

Ferrara G, Bleck B, Richeldi L et al (2008) Mycobacterium tuberculosis induces CCL18 expression in human macrophages. Scand J Immunol 68:668–674

Ferrara G, Losi M, D'Amico R 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

Ferrara G, Losi M, Meacci M et al (2005) Routine hospital use of a new commercial whole blood interferon-gamma assay for the diagnosis of tuberculosis infection. Am J Respir Crit Care Med 172:631–635

Feske M, Nudelman RJ, Medina M et al (2008) Enhancement of human antigen-specific memory T-cell responses by interleukin-7 may improve accuracy in diagnosing tuberculosis. Clin Vaccine Immunol 15:1616–1622

Flynn JL (2004) Immunology of tuberculosis and implications in vaccine development. Tuberculosis 84:93–101

Gaseitsiwe S, Valentini D, Mahdavifar S et al (2008) Pattern recognition in pulmonary tuberculosis defined by high content peptide microarray chip analysis representing 61 proteins from M. tuberculosis. PLoS One 3:e3840

Geluk A, Lin MY, van Meijgaarden KE et al (2007) T-cell recognition of the HspX protein of Mycobacterium tuberculosis correlates with latent M. tuberculosis infection but not with M. bovis BCG vaccination. Infect Immun 75:2914–2921

Hill PC, Jackson-Sillah DJ, Fox A et al (2008) Incidence of tuberculosis and the predictive value of ELISPOT and Mantoux tests in Gambian case contacts. PLoS One 3:e1379

Hoff ST, Abebe M, Ravn P et al (2007) Evaluation of Mycobacterium tuberculosis–specific antibody responses in populations with different levels of exposure from Tanzania, Ethiopia, Brazil, and Denmark. Clin Infect Dis 45:575–582

Höhn H, Kortsik C, Zehbe I et al (2007) MHC class II tetramer guided detection of Mycobacterium tuberculosis-specific CD4+ T cells in peripheral blood from patients with pulmonary tuberculosis. Scand J Immunol 65:467–478

Horsburgh CR (2004) Priorities for the treatment of latent tuberculosis infection in the United States. N Engl J Med 350:2060–2067

Huebner RE, Schein MF, Bass JB Jr (1993) The tuberculin skin test. Clin Infect Dis 17:968–975

Jacobsen M, Mattow J, Repsilber D et al (2008) Novel strategies to identify biomarkers in tuberculosis. Biol Chem 389:487–495

Jafari C, Ernst M, Kalsdorf B et al (2006) Rapid diagnosis of smear-negative tuberculosis by bronchoalveolar lavage enzyme--linked immunospot. Am J Respir Crit Care Med 174:1048–1054

Janssens JP, Roux-Lombard P, Perneger T et al (2007) Quantitative scoring of an interferon-gamma assay for differentiating active from latent tuberculosis. Eur J Resp 30:722–728

Kaufmann SH, Winau F (2005) From bacteriology to immunology: the dualism of specificity. Nat Immunol 6:1063–1066

Khan IH, Ravindran R, Yee J et al (2008) Profiling antibodies to Mycobacterium tuberculosis by multiplex microbead suspension arrays for serodiagnosis of tuberculosis. Clin Vaccine Immunol 15:433–438

Kusner DJ (2005) Mechanisms of mycobacterial persistence in tuberculosis. Clin Immunol 114:239–247

Lalvani A (2007) Diagnosing tuberculosis infection in the 21st century: new tools to tackle an old enemy. Chest 131:1898–1906

Lalvani A, Brookes R, Wilkinson RJ et al (1998) Human cytolytic and interferon gamma-secreting CD8+ T lymphocytes specific for Mycobacterium tuberculosis. Proc Natl Acad Sci USA 95:270–275

Lalvani A, Nagvenkar P, Udwadia Z et al (2001) Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent Mycobacterium tuberculosis infection in healthy urban Indians. J Infect Dis 183:469–477

Leung WL, Law KL, Leung VS et al (2009) Comparison of intracellular cytokine flow cytometry and an enzyme immunoassay for evaluation of cellular immune response to active tuberculosis. Clin Vaccine Immunol 16:344–351

Leyten EM, Arend SM, Prins C et al (2007) Discrepancy between Mycobacterium tuberculosis-specific gamma interferon release assays using short and prolonged in vitro incubation. Clin Vaccine Immunol 14:880–885

Liebeschuetz S, Bamber S, Ewer K et al (2004) Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet 364:2196–2203

Losi M, Bossink A, Codecasa L et al (2007) Use of a T-cell interferon-gamma release assay for the diagnosis of tuberculous pleurisy. Eur Respir J 30:1173–1179

Mazurek GH, Weis SE, Moonan PK et al (2007) Prospective comparison of the tuberculin skin test and 2 whole-blood interferon-gamma release assays in persons with suspected tuberculosis. Clin Infect Dis 45:837–845

Menzies D (1999) Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. Am J Respir Crit Care Med 159:15–21

Mori T, Harada N, Higuchi K et al (2007) Waning of the specific interferon-gamma response after years of tuberculosis infection. Int J Tuberc Lung Dis 11:1021–1025

Mori T, Sakatani M, Yamagishi F et al (2004) Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. Am J Respir Crit Care Med 170:59–64

Morner M (2006) Nya tbc-tester ger säkrare resultat. Smittskydd 3:2006

Nakamura RM, Einck L, Velmonte MA et al (2001) Detection of active tuberculosis by an MPB-64 transdermal patch: a field study. Scand J Infect Dis 33:405–407

Pai M, Zwerling A, Menzies D (2008) Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. Ann Intern Med 149:177–184

Perkins MD, Cunningham J (2007) Facing the crisis: improving the diagnosis of tuberculosis in the HIV era. J Infect Dis 196(suppl 1):S15–27

Reed JR, Vukmanovic-Stejic M, Fletcher JM et al (2004) Telomere erosion in memory T cells induced by telomerase inhibition at the site of antigenic challenge in vivo. J Exp Med 199:1433–1443

Richeldi L, Ewer K, Losi M et al (2004) T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. Am J Respir Crit Care Med 170:288–295

Richeldi L, Losi M, D'Amico R et al (2009) Performance of tests for latent tuberculosis in different groups of immunocompromised patients. Chest 136:198–204

Rieder HL (1999) Epidemiologic basis of tuberculosis control (Paris): International Union Against Tuberculosis and Lung Disease

Rook GA, Stanford JL (1996) The Koch phenomenon and the immunopathology of tuberculosis. Curr Top Microbiol Immunol 215:239–262

Ruhwald M, Bjerregaard-Andersen M, Rabna P et al (2009) IP-10, MCP-1, MCP-2, MCP-3, and IL-1RA hold promise as biomarkers for infection with M. tuberculosis in a whole blood based T-cell assay. BMC Res Notes 2:19

Ruhwald M, Bodmer T, Maier C et al (2008a) Evaluating the potential of IP-10 and MCP-2 as biomarkers for the diagnosis of tuberculosis. Eur Respir J 32:1607–1615

Ruhwald M, Petersen J, Kofoed K et al (2008b) Improving T-cell assays for the diagnosis of latent TB infection: potential of a diagnostic test based on IP-10. PLoS One 3:e2858

Sackett DL, Haynes RB (2002) The architecture of diagnostic research. BMJ 324:539–541

Salgame P (2005) Host innate and Th1 responses and the bacterial factors that control Mycobacterium tuberculosis infection. Curr Opin Immunol 17:374–380

Steingart KR, Dendukuri N, Henry M et al (2009) Performance of purified antigens for serodiagnosis of pulmonary tuberculosis: a meta-analysis. Clin Vaccine Immunol 16:260–276

Steingart KR, Henry M, Laal S et al (2007a) A systematic review of commercial serological antibody detection tests for the diagnosis of extrapulmonary tuberculosis. Thorax 62:911–918

Steingart KR, Ramsay A, Pai M (2007b) Commercial serological tests for the diagnosis of tuberculosis: do they work? Future Microbiol 2:355–359

Thomas MM, Hinks TS, Raghuraman S et al (2008) Rapid diagnosis of Mycobacterium tuberculosis meningitis by enumeration of cerebrospinal fluid antigen-specific T-cells. Int J Tuberc Lung Dis 12:651–657

Vergne I, Chua J, Lee H et al (2005) Mechanism of phagolysosome biogenesis block by viable Mycobacterium tuberculosis. Proc Natl Acad Sci USA 102:4033–4038

Vukmanovic-Stejic M, Agius E, Booth N et al (2008) The kinetics of CD4+Foxp3+ T cell accumulation during a human cutaneous antigen-specific memory response in vivo. J Clin Invest 118:3639–3650

Vukmanovic-Stejic M, Reed JR, Lacy KE et al (2006) Mantoux Test as a model for a secondary immune response in humans. Immunol Lett 107:93–101

World Health Organization (2008) Global Tuberculosis Control Report: surveillance, planning, financing: WHO report 2008 (Geneva, Switzerland: WHO)

Wu B, Huang C, Kato-Maeda M et al (2007) Messenger RNA expression of IL-8, FOXP3, and IL-12beta differentiates latent tuberculosis infection from disease. J Immunol 178:3688–3694