18

Immune Diagnosis of Tuberculosis Through Novel Technologies

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1. Introduction

Due to its highly contagious nature, the control of tuberculosis (TB) is strongly dependent on the efficiency of diagnosis. Natural history of TB comprises two main stages: a latent, non-infective form, in which bacilli are efficiently controlled by the human defense system and, active TB disease, whose pulmonary form is the most common and infective variant in humans (Figure 1). Diagnosis of TB is needed at different stages: the detection of latent TB, a condition estimated to be present in one third of the world population (Russell et al 2010), screening tests for active TB in large populations and confirmatory/drug-susceptibility diagnostic tools aimed to select appropriate chemotherapy regimes. In particular, tools to screen active, contagious TB cases are critical to overcome diagnosis delays, transmission and spread of the disease, and represent one of the top priorities for TB control. For more than a century, diagnosis of active TB has been essentially based on sputum smear microscopy (SSM). Simple and rapid, considered a low-cost control tool, SSM fails however to detect about half of active pulmonary cases since its sensitivity is compromised by low bacterial loads: only above 10^4 bacilli per ml of sputum are detectable by SSM (Abebe et al, 2007). Moreover, detection of positive smears needs to be confirmed in three independent samples so that mycobacteria other than *M. tuberculosis*, which may be present as normal flora do not generate false positive diagnosis results. When suspected cases still remain, time-consuming and high-cost *M. tuberculosis* cultures are required. For many decades, a myriad of alternative tools have been explored to replace SSM for screening active TB. After growth of the pathogen inside a body, exposition to bacterial components is followed by the secretion of specific antibodies. Antibodies associated to active TB are not correlated to protective immunity, but their presence may be exploited as biomarker for active TB. In this chapter, the potential of antigen-antibody measurements to screen active TB will be pointed up, with a special emphasis in the need for controlling particular but large populations. The basis, challenges and opportunities of immune diagnosis will be described, putting an emphasis on our work involving novel technologies.

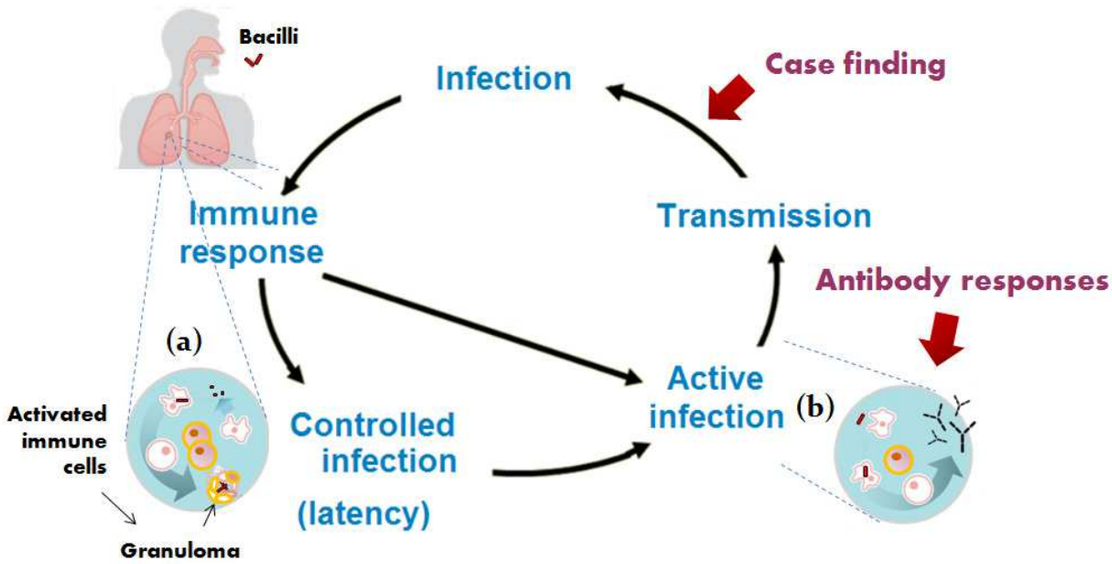

Fig. 1. Natural history of *Mycobacterium tuberculosis* infection. *M. tuberculosis* is an obligate pathogen normally acquired through respiratory tract. After infection, establishment of human response is able to contain microbial growth in 90 – 95% cases; this process is characterized by activation, recruitment and/or proliferation of distinct cells in the infectious foci, growth control and confinement of the pathogen inside a granuloma, a condition called as latent TB (Inlet a). In contrast, immune compromised-associated conditions lead to active disease, characterized by microbial growth, tissue damage and antibody production (Inlet b). Case finding of active cases is critical to stop TB transmission. Antibodies, depicted using the symbol Y, and antigens liberated from bacteria may serve as biomarkers at this infection stage.

2. The need of rapid tests for diagnosing active TB

Efficient treatment is available for most cases of TB. However, thousands of deaths are reported every day due to TB. Due to the fact that healthy immune systems are a condition for TB containment, malnourishment, poverty and a fail in Public Health coverage have been strongly associated to the development of active TB cases. The burdens of TB morbidity and mortality have a tremendous impact in young adults, children and women, and result in the loss of potentially healthy and productive life. It is estimated that active TB cases result in a strong economical impact through loss of work, absence from school and public health expenses. Accentuating this scenario, TB active cases also represent the major cause of death during human immunodeficiency virus (HIV) infection. To deal with this problem, the availability of simple diagnostic tools for the detection of tuberculosis is essential, as it is the basis to treat and control infective cases. During the last years, a set of novel diagnostic methodologies have been developed. Some popular examples are tools based on nucleic acid amplification, such as the polymerase chain reaction (PCR), or the introduction of radioactive probes to speed bacterial culture detection (WHO, 2009). Many new diagnostic technologies had been based on sophisticated equipment and highly specialized training, but up to 90% TB cases occur in low-income settings (WHO 2006),

making those methods unaffordable. Thus, novel approaches must be focused in the diagnosis of TB at high-burden settings. A few years ago, the World Health Organization (WHO) has prompted for the development of tests for active TB optimally fulfilling seven requirements, the so-called ASSURED diagnostic tests:

- Affordable by those at risk of infection
- Sensitive (few false-negatives)
- Specific (few false-positives)
- User-friendly (simple to perform and requiring minimal training)
- Rapid (to enable treatment at first visit and Robust (does not require refrigerated storage)
- Equipment-free
- Delivered to those who need them

Currently, the development of ASSURED tests has been considered for the control of a variety of diseases, including TB, malaria, syphilis and dengue. Considering the number of affected people and the feasibility for its development, the availability of an ASSURED test for the screening of active TB would have a tremendous, positive impact in World Public Health (Mabey et al, 2004). Before the emergence of human immune deficiency virus (HIV) epidemy, TB became "invisible to international donors and taken to be a fact of life in the most-affected parts of the world" (Dye & Williams, 2010). Thereby, the search for better vaccines, therapeutic and diagnostic tools was neglected for decades, even for more than a century in some cases (Kaufmann & Parida 2007). At present, this lack of technological developments has made TB control tools virtually inaccessible for most endemic settings. Manipulation of *M. tuberculosis* specimens represents a high level biological risk, requiring high-cost, sophisticated facilities. For these reasons, diagnosis based on the immune response to *M. tuberculosis* represents an alternative to cover the main indications for an ASSURED test, including minimal handling requirements, rapidity and adaptability to close-to- the-patient formats. In this chapter we will describe the biological basis, challenges and opportunities related to immunological tests, with an emphasis on point-of-care (POC) tests. In addition to rapid immunochromatography formats that have been explored by various groups, we herein propose the development of friendly, label-free platforms, using Micro-Electro-Mechanical Systems (MEMS).

3. Human immune response to *Mycobacterium tuberculosis* infection

It is estimated that one third of the World population is currently infected by *M. tuberculosis* (Dye & Williams, 2010). After infection, *M. tuberculosis* is promptly recognized by the innate immune system. Pattern-recognition receptors located at the surface of myeloid cells, such as mannose recognizing receptors located in macrophages or dendritic cells, recognize molecular patterns commonly associated to pathogens and immediately respond through pro-inflammatory signals (Dorhoi et al, 2011). Whether this process is able to control the spread of the bacilli is still controversial. Supporting the hypothesis is the fact that a number of healthy contacts have been reported with no apparent sign of acquired immunity, thus suggesting that innate mediators were able to stop the infection before the establishment of adaptive, memory-derived responses (Dorhoi et al, 2011). In most people, however, the establishment of innate immunity allows a set of specific defence mechanisms to be

initiated. This process involves destruction of bacilli by professional phagocytes, and presentation of pathogen molecular fragments (antigens) to lymphocytes, followed by activation and proliferative steps. This response, also known as adaptive immunity, may give rise to either protection via the containment of the bacilli inside a granuloma, or an exacerbated inflammatory process associated to destructive pathology. In both cases, lymphocytes that specifically recognize their cognate antigen are activated, leading to the production of cytokines or antibodies, the destruction of infected cells and, the formation of memory lymphocyte clones. Since these processes involve mechanisms specifically originated during *M. tuberculosis* infection, the associated biomarkers have been largely exploited for diagnostic purposes.

An overview of adaptive, cell-mediated responses to *M. tuberculosis* is schematized in Fig. 2. Adaptive responses take place when lymphocytes recognize mycobacterial molecules as

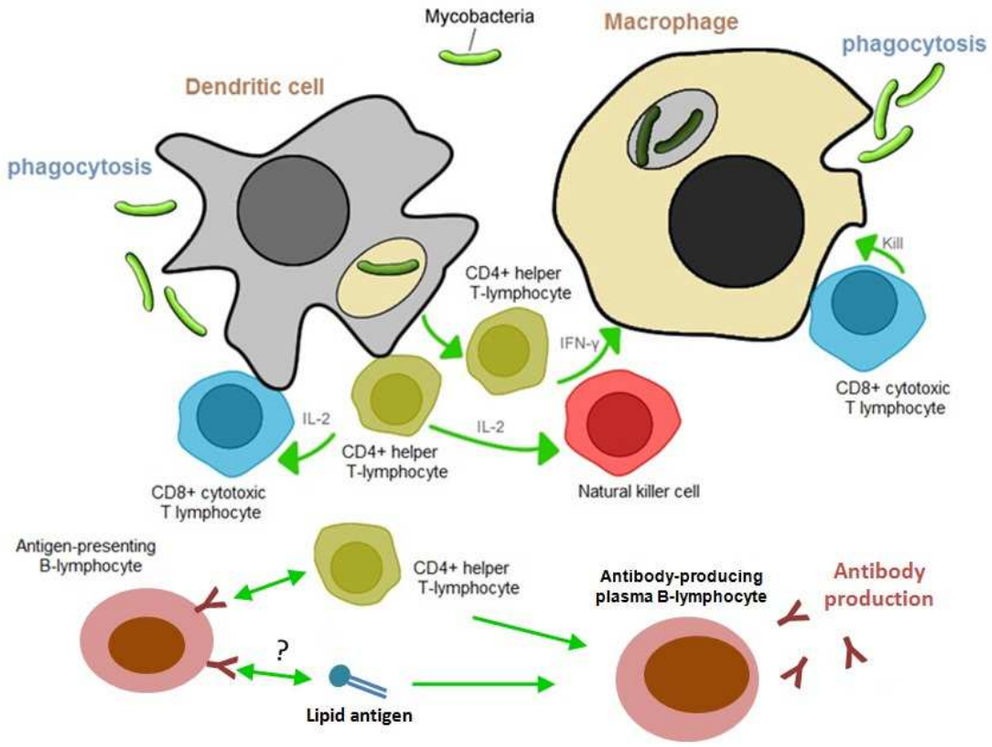

Fig. 2. Cell-mediated immunity against *M. tuberculosis* infection. After recognition and engulfment of the pathogen by phagocytic cells (macrophages, dendritic cells), bacterial components (antigens) are processed into small fragments and presented to lymphocytes. Thymus-derived T-lymphocytes recognize antigens presented by antigen-presenting cells, such as macrophages, dendritic cells and B-lymphocytes. After antigen recognition, T-cells are activated and develop into cytokine-producing (CD4+) cells or cytolytic (CD8+) mediators. B-cells are directly activated by antigens. However, fully activation of B-cells may be coordinated by interactions with CD4+ T-cells, in the case of peptide antigens, or through thymus-independent, poorly described pathways for non-protein antigens, such as lipids. Figure is out of scale.

foreign entities (as antigens). Two lymphocyte families, the T and B-cells, are activated after encountering with their matching antigen, then develop into the effectors of adaptive immunity. For T cells, presentation of foreign entities by antigen-presenting cells is mandatory for recognition. In contrast, B cells recognize their cognate antigens through direct interaction via the B-cell receptor. Once activated, B and T cells trigger a variety of functions, mainly including: (a) secretion of cytokines and chemoattractants by CD4+ helper T-cells, (b) lysis of infected cells via the release of lytic enzymes by CD8+ cytotoxic T-cells, (c) secretion of antibodies by plasma B-cells that have been derived from activated B-lymphocytes, (d) production of a number of long-lived memory T- and B-cells, which last for many years circulating in the bloodstream and monitor for infection (either newly developed or endogenously re-activated).

As depicted in Figure 2, some responses related to cell-mediated immunity are restricted to active infection. One illustrative example is the release of some pro-inflammatory cytokines by T-cells, such as interferon-gamma. The secretion of specific antibodies by plasma B-lymphocytes also occurs during active or newly acquired TB, where antigen-presenting cells are charged with bacterial moieties. Some of the immune responses during active infection are known to be critical for arresting the growth of *M. tuberculosis*. In contrast, antibody secretion has been considered irrelevant, although some recent studies suggest a role for antibodies on the fate of *M. tuberculosis* infection (Glatman-Freedman et al, 2010). Yet, secretion of antibodies during bacterial growth (*i.e.* during active infection) can be exploited to detect infectious cases, thus contributing to stop TB transmission.

Isolated specific antigens have been used for detecting antibodies in human plasma, through the measurement of antigen-antibody reactions *in vitro*. Alternatively, antibodies may be produced in the lab and used to capture mycobacterial antigens that have been secreted to body fluids. Because free mycobacterial antigens are not encountered in many biological fluids, our work has been dedicated to the detection of antibodies. Importantly, antibodies associated to *M. tuberculosis* disease are found in sera from individuals affected by both pulmonary and extra-pulmonary TB, thus allowing the detection of difficult-to-detect TB pathologies (Daniel, 1989). Challenges related to the production of antigens for diagnostic tests will be presented in the following sections.

4. Antigen repertoire of *M. tuberculosis*

Specific activation of immune cells against *M. tuberculosis* occurs after the recognition of bacterial components as foreign entities. With about 4,000 genes (TubercuList web page), *M. tuberculosis* synthesizes a complex array of molecular products, mainly composed of proteins, lipids and carbohydrates. Box 1 summarizes the highlights of different antigens from the bacillus.

5. Immunodiagnosis of TB: From Koch to POC tests

A few years after Robert Koch discovered *M. tuberculosis*, his work was largely dedicated to look for a cure against TB disease, which was the major health threat in Europe by the time. His work allowed the description of *M. tuberculosis* extracts, obtained by glycerol extraction of liquid cultures of the bacilli (Kaufmann & Schaible 2005). Although this material, called tuberculin, was found unable to inhibit the growth of *M. tuberculosis* in guinea pigs or

***M. tuberculosis* is a bacterial pathogen of atypical molecular composition:**

- Protein molecular patterns include stress-inducible proteins of wide distribution within bacteria, enzymes presenting homology with many other human pathogens (bacteria and parasites), and newly described or putative gene products devoid of homology vis-à-vis any other peptide annotated so far (16% of *M. tuberculosis* open reading frames are novel sequences).
- Cellular wall is unusually thick, conferring unique properties of tinction (*M. tuberculosis* is not Gram+ nor Gram-) and atypical antibiotic susceptibility patterns.
- Genes related to lipid metabolism are especially abundant (5-fold more genes in *M. tuberculosis* genome compared to that of *Escherichia coli*). In accordance, the Koch bacillus produces lipids with amazing structural features and sizes (fatty acids up to 80 carbons length). Many of these lipids are recognized as antigens during active TB.
- Carbohydrate products have distinctive structures. Polysaccharides and glycoconjugates (glycolipids, glycoproteins) comprise molecular motifs recognized by innate immune cells and important virulence factors. In contrast to findings in model animals, sera from *M. tuberculosis*-infected humans strongly recognize sugar structures.
- Shared with other bacteria, some small phosphorus-containing compounds, phosphoantigens, constitute a separate group of antigens in *M. tuberculosis*. Phosphoantigens activate a set of unusual T-cells and possess interesting therapeutic effects against specific lymphomas.

Box 1. **Antigens from *Mycobacterium tuberculosis***

humans, Koch reported for the first time that previously-infected individuals developed a local inflammation at the site where tuberculin was injected, whereas healthy controls did not present such a response (Kaufmann & Schaible 2005). This hypersensitivity reaction was later related to the activation of memory T-cells, which are able to recognize tuberculin-containing antigens. Named the delayed-type hypersensitivity (DTH) test, the intradermal reaction to tuberculin constituted the first diagnostic tool based on the immune response against *M. tuberculosis*, and has been used for more than a century to diagnose latent TB (WHO 2006). During latent TB, memory T-lymphocytes may be searched through either DTH tests performed *in-vivo*, or more rapid, *in-vitro* cytokine analyses (Lalvani 2007, Hanekom et al, 2004). In any case, crude mixtures of mycobacterial antigens, such as tuberculin, often produce unspecific, false-positive results. In fact, *M. tuberculosis* shares a number of antigens with other microorganisms, including vaccine strains against TB. To circumvent this problem, the use of single or a small cocktail of antigens has been proposed. Two secreted protein antigens, ESAT-6 and CFP-10, are worth of mention since a high specificity and abundant T-cell responses have been associated to them (Fox et al, 2007). More recently, a non-protein, lipid antigen has also been proposed as a reagent to look for DTH responses (Komori et al 2011). Considering the lack of gene polymorphism of lipid-presenting molecules in humans (De Libero & Mori 2010), introduction of lipid antigens for diagnosing latent TB appears very promising.

In contrast to memory T-cell responses, production of antibodies requires the presence of plasma circulating B-lymphocytes, a phenomenon associated to active infection (see Figure 2). Therefore, the search for antigen-antibody reactions has been largely explored to diagnose active TB. Such a reaction may be measured in a wide set of platforms, from old

agglutination and electrophoretic immune precipitation techniques (Zykov et al 1966), to lab-on-chip formats, in which a set of laboratory procedures are automatically performed within a microfluidics technology-based chip (Schulte et al 2002). Contrasting to cell-mediated responses, the assessment of antigen-antibody reactions does not require special conditions, such as regulated temperature or specific environment. In addition, no exposition to high level biological risk bacilli is involved to obtain plasma or serum samples.

The search for antibodies in body fluids may be performed using a variety of technological platforms. Currently, Enzyme-Linked ImmunoSorbent Assay (ELISA) is the most common method for analyzing antigen-antibody reactions. The typical format used for screening antibodies in sera, an indirect ELISA, is schematized in Figure 3. ELISA has been widely used since the late 80's to screen for active TB (Daniel, 1989). Crude bacterial extracts, including tuberculin, represented the first bacterial materials explored as reagents. One of the most popular mixtures was Antigen-5, a preparation composed of various proteins and lipoarabinomannan, a specific cell-wall glycolipid (Daniel, T. M. et al, 1985). As modern Biochemistry tools have been developed, more purified, specific antigens have been obtained. Up to now, many proteins, post-translationally modified peptides, glycolipids and saccharides have been proposed as antigens for immunodiagnosis of active TB (Steingart et al, 2009).

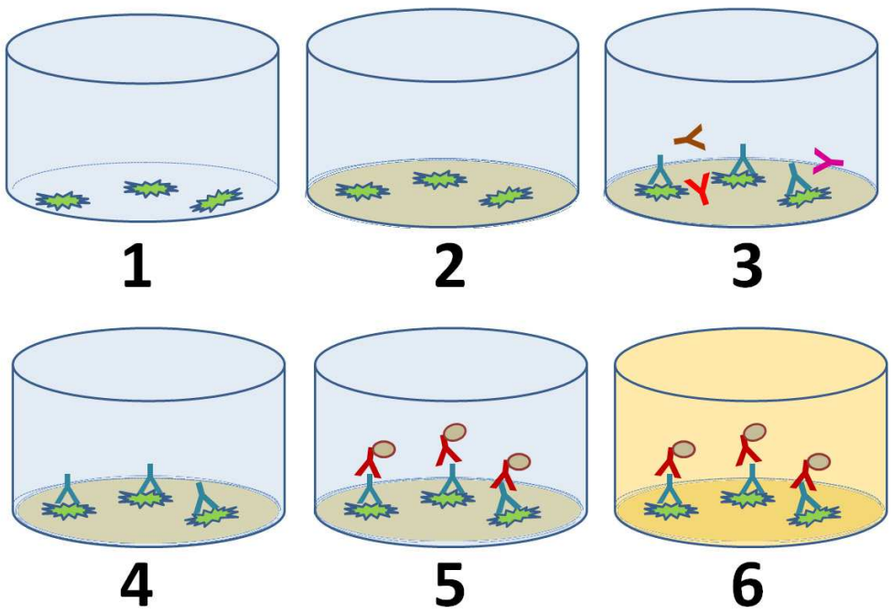

Fig. 3. The Enzyme-Linked ImmunoSorbent Assay for the detection of antibodies. A selected antigen is used to coat a plate surface (1); a solution of non-reacting protein, such as bovine serum albumin, is used to block non-occupied sites at the plate surface, avoiding non-specific binding between serum proteins and the plate (2); serum is added and antibodies bind to matching epitopes of immobilized antigens (3); non-reactive antibodies from sera are eliminated by washings (4); a secondary antibody linked to an enzyme is used to bind the primary, tuberculosis-associated antibody (5); the substrate of the enzyme is added allowing to enzyme-dependent colorimetric changes, which are quantified through spectrophotometry (6).

The development of ELISA represented a breakthrough for the detection of antigen-antibody responses. Before enzyme-linked tests, cumbersome and/or hazardous methods, including radioimmunoassays (RIA), importantly limited the application of immune diagnostic tests for large populations (Lequin, 2005). Yet, an ELISA presents important limitations for large-scale applications. Major drawbacks associated to this technique include: the need of large amounts of samples, a multistep procedure poorly suitable for high throughput scales, and high costs related to the use of enzymatic markers, specialized equipment and trained personnel. Recently, the development of more friendly, equipment-free technologies has been largely explored, importantly including the search for miniaturized analytical devices. In particular, the integration of currently existing detection methods, such as those based on antigen-antibody responses, into microdevices represents a promise for bringing diagnostics closer to those who need it. The introduction of microtechnologies may afford automated systems for the detection of TB at the site of patient care. Such devices have been called Point-Of-Care (POC) tests.

6. Current challenges for the development of POC tests for TB diagnosis

TB is considered one of the most complex diseases ever established in humankind. Active TB has been related to multiple processes but not to any single pathogenic or host factor. Therefore, the demand of technological innovations for the development of POC tests is accompanied by a particular challenge regarding the selection of bioreagents. In the following paragraphs, we enumerate the major current limitations and possible technological solutions for the development of POC tests to diagnose active TB.

6.1 The heterogeneous human response to *M. tuberculosis* antigens

Up to now, few systematic studies to address the antibody response during tuberculosis in humans have been performed. The presence of different antibody secretion patterns in humans has been largely observed. HLA genes encoding for molecules presenting protein antigens, namely the Major Histocompatibility Complex (MHC) molecules, are known to be highly polymorphic in humans, and some data indicate this gene polymorphism as a source of variability to recognize peptide motifs by immune cells (Bothamley et al, 1989). On another side, the spectrum of responses at different stage of the disease may account for important variability. In this way, protein antigens that had proved high sensitivities in some trials, have given unsatisfactory results when tested in a different setting (Gennaro, 2000). The use of species-specific antigens, not present in mycobacteria other than *M. tuberculosis,* is known to be required to avoid false-positive results, and antigens synthesized by vaccine strains are also precluded. According to a meta-analysis reported by Steingart et al. (2009), antibody detection methods could achieve high efficiencies only if a mixture of multiple antigens is used. Most analyzed trials failed, however, to include appropriate healthy controls, thus limiting the results in regard to test specificity. Therefore, in spite of the need to perform better diagnostic trials including appropriate controls, a conclusive remark is the convenience to use a cocktail of antigens. With this in mind, antibodies associated to active TB seem largely elicited by unusual, difficult to obtain mycobacterial antigens.

6.2 The structural nature of various sensitive, specific antigens is not addressable via recombinant technology

According to serodiagnostic analyses performed in TB endemic settings (including ours), antigens able to achieve high diagnostics performances represent mycobacterial components non-addressable by genetic engineering. Some of the highest specificities and sensitivities have been found using glycolipids. These are fatty acyl-containing carbohydrate, secondary genetic products, whose biosynthesis involves multiple enzymatic steps, some of them still undefined. Lipoarabinomannan (LAM), di-*O*-acyl trehalose (DAT) and cord factor (CF) belong to this group of difficult-to-obtain highly performance antigens (Barihuta et al, 1993; Escamilla et al, 1996; López-Marín et al, 2003; Maekura et al, 1993; Julián et al, 2001; Simonney et al, 1996). Besides, some of the best protein antigens to diagnose active TB are post-translationally modified products, namely glycosylated proteins such as the 38-kDa, antigen (Espitia et al, 1989). Worth noting, the best diagnostic performances obtained with this protein have been found when it has been obtained from glycosylating mycobacterial cells. In contrast, a non-glycosylated 38-kDa antigen obtained by recombinant technology in *Escherichia coli* has shown poor efficiencies (Gennaro, 2000).

6.2.1 Surrogate microbial sources of secondary-genetic products

The isolation of glycosylated antigens involves cumbersome steps, making difficult their use in large-scale applications. The involvement of complex, methyl-containing fatty acyl structures in antibody recognition may limit the use of synthetic approaches for these antigens. In view of the structural similarity of glycolipids from *M. tuberculosis* and some other mycobacterial species, we looked for surrogate sources of glycolipids as antigens for TB diagnosis. Structural and serologic studies of glycosylated lipids from mycobacteria allowed the identification of *Mycobacterium fortuitum*, a species of rapidly growing non-tuberculous mycobacteria, as surrogate source for two promising antigens: di-*O*-acyl trehalose (DAT) (Escamilla et al, 1996), and cord factor (López-Marín et al, 2003). Interestingly, *M. fortuitum* synthesizes abundant quantities of DAT and cord factor. The antigens afford specific reactivities vis-à-vis healthy controls and patients infected with other pathogenic actinomycetes (López-Marín et al, 2003). In addition, glycolipids from *M. fortuitum* are not longer recognized by individuals with healed TB and do not present cross-reactivities with vaccinated healthy controls (Escamilla et al, 1996).

6.2.2 Peptide mimicry and combinatorial strategies

Glycosylation has been detected as a critical factor for antibody recognition during active TB. For instance, the ability of antigens from *M. tuberculosis* to bind antibodies in sera from infected people is strongly decreased after periodate treatments, indicating that antibodies predominantly react with carbohydrate determinants (Udaykumar & Saxena, 1991). Sugar antigens in *M. tuberculosis* comprise protein and lipid glycoconjugates. At present, recombinant production of mycobacterial sugars is unfeasible, since biosynthetic pathways are still poorly described. A few decades ago, phage-display based technologies emerged as a powerful method to look for structurally diverse unknown ligands. Through recombinant technology, *E. coli* phages are modified to obtain combinatorial peptides libraries displayed on the virion surface (Smith, 1985). Through this technology, phage displayed peptides with affinity to any ligand can be identified by in-vitro screening (Figure 4). Using a phage

displayed dodecapeptide library, we have selected phages with specific binding to a serum directed towards *M. tuberculosis* carbohydrate antigens (Gevorkian et al, 2005). This approach resulted in the identification of peptides that mimic mannose-containing molecules of *M. tuberculosis*. A set of peptides were readily recognized by antibodies raised against mycobacterial sugars. More surprisingly, one of these peptides induced, in rabbits, the production of antibodies recognizing mannan. More recently, Bua and coworkers used this technology to obtain phages as useful reagents for the serodiagnosis of tuberculosis (Bua et al, 2009).

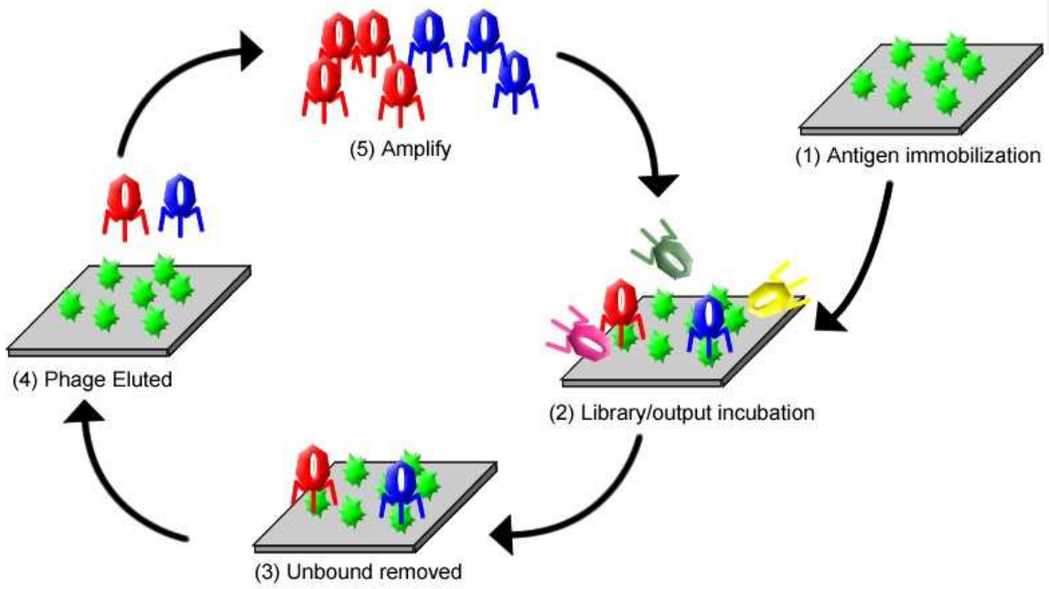

Fig. 4. Phage Display technology for the identification of biological reagents. Combinatorial peptide libraries displayed in virion surfaces can be obtained through genetic engineering. These libraries have proved to be useful for the identification of antibody ligands, even for those associated to poorly defined antigens. In this method, antigens are immobilized on a plate (1) and incubated in the presence of phage suspension (2); non bound phages are removed by washings (3), and selected phage clones are obtained through pH-mediated elution (4); finally, selected clones are amplified (5). The selection cycle is usually repeated 3 times.

Oligopeptides have been found to mimic a set of 3D structures, including sugars, linear and conformational peptide epitopes. Therefore, phage display represents a promising technology for the identification of peptides able to replace difficult-to-obtain or previously unknown antigens. At present, our work is focused to find optimal conditions in order to use peptide mimotopes as immunodiagnostic reagents.

6.2.3 New vectors for the recombinant production of post-translational modified antigens

A different approach to get reagents for diagnosis of TB has been the production of glycosylated proteins of *M. tuberculosis* in novel expression systems. Traditionally, recombinant mycobacterial proteins have been produced in *E. coli*. Mycobacterial glycosyl

structures are distinct from mammalian carbohydrates. Thus, expression of *M. tuberculosis* glycoproteins in other actinomycetes, such as rapidly growing *Mycobacterium smegmatis* (Garbe et al, 1993) and *Streptomyces* (Lara et al, 2003) appears as an interesting tool towards the application of serodiagnostics.

6.3 Currently available platforms for POC immunodiagnostics

Screening tests to detect TB are essential to overcome the epidemic. In particular POC tests may be implemented at lower levels of health services, contributing to stop TB transmission. The use of microfluidic technologies seem of special interest since they are associated to small volumes of samples. For instance, POC tests have been already developed to monitor biomarkers in a few blood drops. Two platforms for the study of antigen-antibody interactions are described below, immunochromatography tests and microchip-based devices.

6.3.1 Lateral-flow immunochromatography tests

One of the most advantageous platforms for the study of antigen-antibody reactions is lateral-flow immunochromatography. The basis of this method is schematized in Figure 5. Immunochromatography tests enable added value to antigen-antibody reactions since they allow higher throughput, reduced volume of samples, as well as lower costs than traditional immunoassays, since no specialized equipment or skilled personnel are necessary. Although this format has been used in many endemic settings, antigen evaluations vis-à-vis healthy controls or related pathologies require further studies. To our knowledge, highly specific glycolipid antigens, hydrophobic in nature, have not been included in immunochromatography formats.

6.3.2 Micro-Electro-Mechanical Systems (MEMS)

Based on resistant but flexible silicon platforms constructed through microchip-based technologies, MicroElectroMechanical Systems (MEMS) are micrometric devices which include mechanical parts, such as actuators, sensors or integrated microfluidic systems. MEMS have been widely used in aerospace and automotive industry. Examples of some popular MEMS are microaccelerometers in crash air-bag systems or micromirrors for projection systems. Some of the most attractive features in MEMS are reliability and low-cost, which are associated to their large batch processing. Interestingly, a MEMS is able to perform automated analyses, including transport, separations, chemical reactions and sensing. All necessary instruments can be integrated in a single device, the MEMS, so that new terms, micro Total Analysis Systems (μTAS), and "Lab-On-a-Chip" (LOC) technologies have been coined for such instruments. Over the course of the past fifteen years, MEMS have also been explored for a set of biomedical applications, including metabolite analyses, drug testing, drug discovery, combinatorial assays for DNA screening and, obviously, immunodiagnosis (Hedlund, 2009). Figure 6 shows a scanning electron micrography of a MEMS. A detailed description of MEMS is out of the scope of this chapter. However, we enumerate the key features allowing this technology to be a promising tool for the development of ASSURED tests for diagnosis (Box 2).

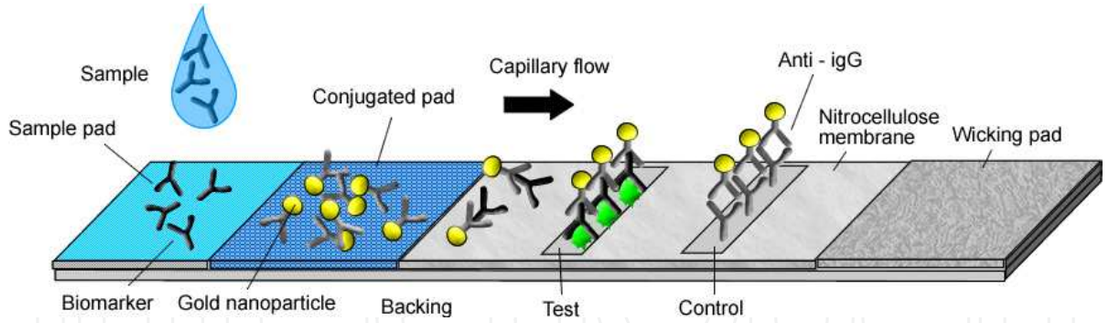

Fig. 5. Immunochromatography platform for assessment of antigen-antibody reactions. Immunochromatography strips contain a sample pad, in which biological fluids are deposited. Capillarity induces a lateral flow of the sample throughout the pad. Frequently, nanocolloidal gold particles are functionalized for conjugation to total antibodies. Antigen immobilized as a line in the nitrocellulose strip captures the antibody-colloidal gold complex. A control line reacting with colloidal gold is also present. After developing, the test can be read since purple lines develop if colloidal gold is hold.

To illustrate the suitability of MEMS technology for clinical POC tests, a good example is the possibility to sense biological markers in a sample using label-free technologies. In MEMS, physical properties, such as conductivity or mass changes, are usually analyzed through electric or optical low-cost systems (Battiston et al, 2001; Fischer, 2011). For immune diagnostic systems, the capture of antibodies by antigen-displaying surfaces will result in changes of mass or conductivity parameters, both of them addressable through MEMS standard technologies (Figure 7).

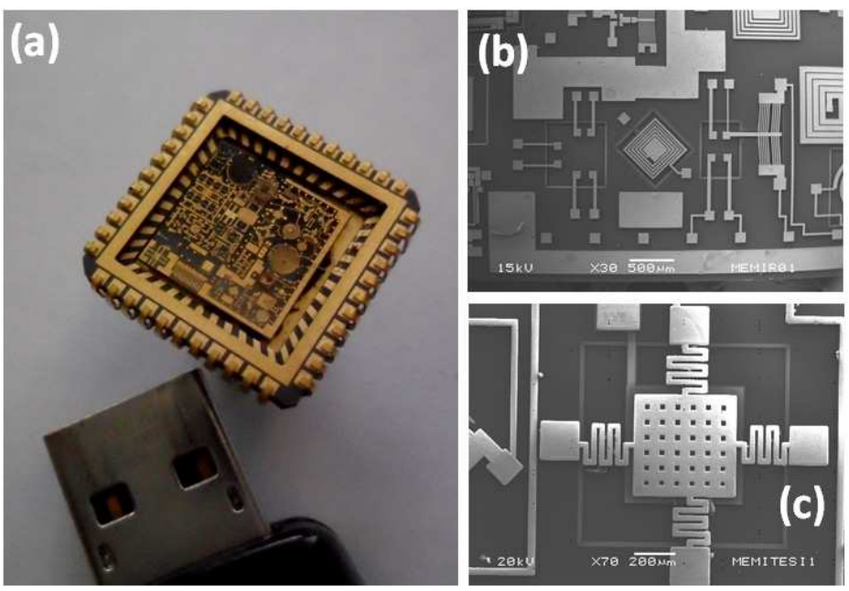

Fig. 6. Panoramic image (a) and scanning electron micrographs (b, c) of a MicroElectroMechanical System (MEMS). Constructed using microchip technology, such as lithography on silica supports, MEMS also contain mechanical components, including beams, gears, diaphragms, grooves, orifices, springs or suspensions and optical systems. Three dimensional fabrication processes allow the design of automated systems able to reproduce any laboratory procedure.

- MEMS are machines with sizes ranging from a micrometer to a few milimetres
- Including mechanical and optical parts, such as actuators, motors or sensors, MEMS are automated Lab-On-Chip machines
- Batch processing determines very low-costs for an individual machine.
- A variety of materials in MEMS technology, including some new advanced materials make possible the application of MEMS in Biomedicine
- Metal surfaces able to immobilize a set of molecules, by keeping bioactivity
- Some developments for biomedical applications already follow clinical tests for their introduction to the market. These include bioMEMS to sense glucose during diabetes or antibodies levels to detect dengue

Box 2. MicroElectroMechanical Systems (MEMS) at a glance

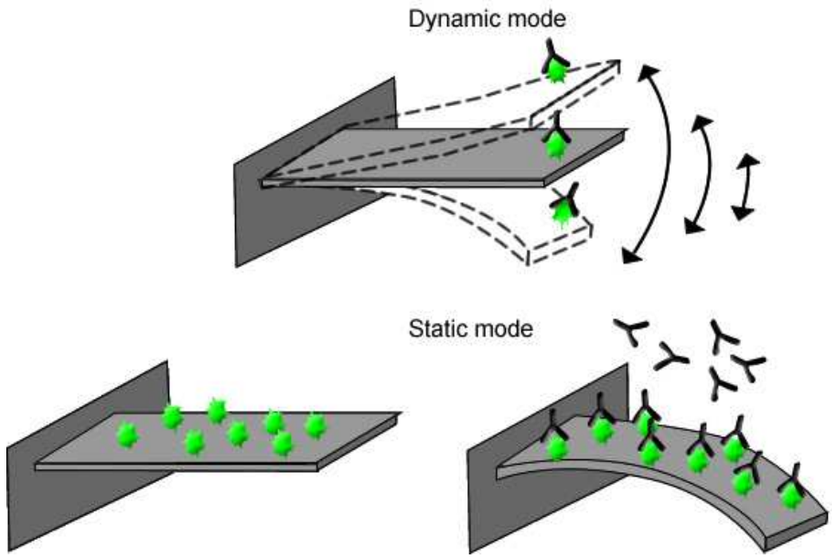

Fig. 7. Label-free measurement systems in MEMS include cantilevers. If antigens are immobilized onto a quartz crystal, fine mass changes, as those produced by antibody capture, will result in measurable alterations in the quartz resonance frequency, an optical property that can be precisely detected combining the use of a laser microbeam and a photodetector

7. Conclusion

A rapid and affordable test for detecting contagious TB patients is the cornerstone of current strategies for TB control. Although a set of clever methods has been proposed for this objective, the search for new tools adaptable to low-resource endemic settings is still a demand. The search of antigen-antibody responses for diagnosing active TB represents a promising alternative, since the associated methods may fulfill the major diagnostic requirements, namely minimal handling, rapidity and adaptability to point-of-care formats. Future success of immunodiagnosis tools for detecting TB will depend on both basic and technological advances: (1) The identification of specific, affordable biological reagents for large scale production (antigens, antibodies or fragments) and (2) The development of immunological tests into low-cost, friendly formats. According to different studies

addressing antigen-antibody responses in different populations, the search for antibodies in TB patients must include specific peptides, but also non-protein (lipid) and post-translational modified antigens, such as glycosylated proteins. In this regard, strategies to obtain complex antigens are critical. The development of novel expression systems and phage-display technologies could be the answer. Finally, we herein suggest that, in addition to further improvements of the already explored immunochromatography strips for TB diagnosis, Micro-ElectroMechanical Systems (MEMS) deserve a particular attention to develop better POC tests for diagnosing active, but also to look for tests addressing the detection of inactive, latent TB.

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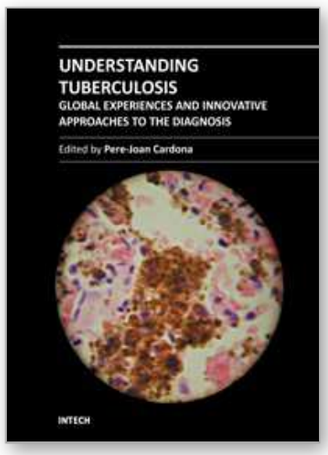

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Mycobacterium tuberculosis is a disease that is transmitted through aerosol. This is the reason why it is estimated that a third of humankind is already infected by Mycobacterium tuberculosis. The vast majority of the infected do not know about their status. Mycobacterium tuberculosis is a silent pathogen, causing no symptomatology at all during the infection. In addition, infected people cannot cause further infections. Unfortunately, an estimated 10 per cent of the infected population has the probability to develop the disease, making it very difficult to eradicate. Once in this stage, the bacilli can be transmitted to other persons and the development of clinical symptoms is very progressive. Therefore the diagnosis, especially the discrimination between infection and disease, is a real challenge. In this book, we present the experience of worldwide specialists on the diagnosis, along with its lights and shadows.

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