REVIEW ARTICLE

Innovative Strategies to Diagnose and Monitor Tuberculosis Patients

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***Mycobacterium tuberculosis* is the single most deadly microorganism worldwide. A third of the world population is thought to have latent tuberculosis and approximately 2 million people die of the disease each year. Short and closely supervised treatment regimens are needed, but it is also essential to develop new strategies to ensure prompt diagnosis of the disease. In particular, cheap methods are needed to tackle tuberculosis from a population perspective. The present article reviews the advances in immunology and molecular strategies for epidemiological diagnosis and monitoring of tuberculosis patients.**

Key words: Mycobacterium tuberculosis. *Mimotopes MIRU-VNTR Hsp65. IS6110. Diagnosis.*

Estrategias innovadoras para el diagnóstico y seguimiento de los pacientes tuberculosos

***Mycobacterium tuberculosis* es el microorganismo que por sí solo ha ocasionado el mayor número de muertes a escala mundial. Se estima que la tercera parte de la población mundial presenta tuberculosis latente, y cada año mueren alrededor de 2 millones de personas en todo el mundo. Además de la aplicación rigurosa del tratamiento acortado y estrictamente supervisado, es imperativo el desarrollo y aplicación de nuevas estrategias que permitan el diagnóstico oportuno de la enfermedad, particularmente de métodos de bajo coste que permitan abordar la problemática de la tuberculosis desde una perspectiva poblacional. En el presente trabajo se revisan los avances en las estrategias inmunológicas y moleculares para el diagnóstico y seguimiento epidemiológico de los pacientes tuberculosos.**

Palabras clave: Mycobacterium tuberculosis. *Mimótopos. MIRU-VNTR. Hsp65. IS6110. Diagnóstico.*

Introduction

Mycobacteria (that is, species of the genus *Mycobacterium*) form a group of just over 100 weakly gram-positive species. Although most of these species are saprophytic, some are pathogenic for humans and other animals. All the species of the genus have an increased resistance to acid environments thanks to a complex cell wall extraordinarily rich in lipids. The nonpathogenic species grow quickly whereas the pathogenic ones are characterized by their slow growth, with generation times ranging from 12 to 24 hours.

The species that cause natural disease in humans or animals belong to either the *Mycobacterium tuberculosis* complex (*M tuberculosis, Mycobacterium bovis, Mycobacterium canetti, Mycobacterium africanum*, and *Mycobacterium microti*) or the *Mycobacterium avium–intracellulare* complex (*M avium* subsp *avium, M avium* subsp *paratuberculosis, M avium* subsp *silvaticum*, and *M intracellulare*). Of these mycobacterial species, *M tuberculosis* is without doubt the most important obligate pathogen responsible for tuberculosis in humans, followed by *M bovis,* whereas *M avium* subsp *avium* and *M intracellulare* can cause pulmonary disease in immunosuppressed subjects. It is estimated that up to 70% of patients with acquired immunodeficiency syndrome ($CD4^+$ ≤50/μL) caused by the human immunodeficiency virus are infected by at least one of these opportunistic pathogens.[1]

Pulmonary tuberculosis is the most common form of the disease and also the most important one in epidemiological terms. The disease is contagious and chronic, and affects humans of all ages. It is estimated that a third of the world population is infected with *M tuberculosis* and, according to official figures published by the World Health Organization, there are approximately

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8 million new cases of tuberculosis and 2 million people die of the disease each year.[2] Despite widespread availability of the bacillus Calmette–Guerin (BCG) tuberculosis vaccine, the incidence of the disease has increased. Moreover, the effectiveness of this vaccine has been questioned,[3,4] although vaccinated individuals are clearly less likely to develop extrapulmonary forms of the disease, and children in particular are afforded protection against meningeal tuberculosis.[5,6]

In Mexico, according to official health ministry figures, tuberculosis is the 17th most common cause of death among the general working population. Nevertheless, as in the rest of the world, *M tuberculosis* is the single most deadly microorganism. In Mexico, 7257 new cases of pulmonary tuberculosis and 76 new cases of meningeal tuberculosis had been reported through to week 28 of 2006[7]; the World Health Organization reports a prevalence of 53 cases per 100 000 inhabitants throughout the Americas.[2]

Traditionally, pulmonary tuberculosis is clinically suspected in individuals with chronic cough and radiological evidence of pulmonary lesions, which are most frequently located in the apical region of the right lung. Once all the clinical criteria have been assessed, diagnosis with laboratory tests continues with sputum smears to assess whether acid-fast bacilli are present.[8] Definitive diagnosis is made by culturing and identifying the infectious species from bronchial secretions of the patient.[9] A wide range of diagnostic strategies of varying diagnostic value are currently available (Table 1). Even without including the time necessary to test for sensitivity to antimicrobials, microbiological diagnosis of tuberculosis requires 6 to 8 weeks—a long delay before starting treatment with antituberculosis agents. On the other hand, prescription of drugs without waiting for the in vitro sensitivity results and lack of treatment compliance have favored the appearance of multidrug–resistant strains of *M tuberculosis.* It is also important to note that factors other than diagnosis of infection, such as lack of access to health services and prompt medical care, also undermine attempts to control the pandemic.[10]

Short and closely supervised treatment regimens are needed, but it is also essential to develop new strategies to ensure the disease is diagnosed promptly. In particular, cheap methods are needed to tackle tuberculosis from a population perspective. Below, we present the most promising strategies being developed for diagnosis and epidemiological surveillance of tuberculosis. These can be classed as: *a)* immunological methods that detect circulating antibodies in the patient (serologic diagnosis), and *b)* molecular methods that aim to identify and genotype clinical isolates of *M tuberculosis.*

Immunological Methods

The immunological methods most commonly studied for diagnosis of tuberculosis are based on detecting serum antibodies against mycobacterial antigens, which can be proteins, lipids, or polysaccharides. Alternatively, specific mycobacterial antigens can be identified in secretions of patients using ex vivo generated monoclonal antibodies.

Tests Based on Antibody Detection

Analyses based on detection of antibodies against *M tuberculosis* are an important alternative to traditional methods for diagnosis of active tuberculosis because they can detect immune response induced during infection. This response is either not present or reduced in asymptomatic infected subjects (that is, those with latent tuberculosis). Furthermore, the type of immunoglobulin (Ig) detected (IgG or IgM) could indicate whether the infectious process is progressing or not. Serologic methods also have the advantage of being easy to do, cheap, and noninvasive, but they are limited in that there is a lack of highly specific and sensitive antigens, as discussed below.

In the enzyme-linked immunosorbent assay (ELISA), or a simplified form thereof (Dot-ELISA), *M tuberculosis* antigens are adsorbed onto a solid phase where they capture specific mycobacterial antibodies from serum samples. Antibody capture is detected with a second antibody conjugated to an enzyme (often peroxidase), which reacts with its substrate (hydrogen peroxide in the case of peroxidase) so indicating when antibody-antibody reaction has taken place. Unfortunately, this test cannot be used with whole protein extract from the bacillus in systematically vaccinated populations (such as in Mexico) because cross reactions occur with antigens in the vaccine strain (*M bovis*-BCG) and sometimes even with saprophytic mycobacteria found in abundance in the environment.[11,12,13]

Protein Antibodies

Antigens purified from *M tuberculosis* have been used as an alternative to limit cross reactivity. However, the humoral response to most of the immunodominant antigens, although strong, varies greatly among tuberculosis patients,[14] and so no antigen is systematically recognized by everyone. A sensitivity of around 80% in patients with positive sputum smears can be achieved using the 38-kDa protein, which is one of the most promising antigens for serodiagnosis. For patients with negative sputum smears, however, the sensitivity is as low as 15%.[15] Thus, one of the main challenges is to design a strategy for rapid diagnosis of pulmonary tuberculosis in patients with

TABLE 1
Characteristics of the Main Methods for Diagnosing Tuberculosis

Test	Time Required	Main Drawbacks	Diagnostic Value
Sputum smear	3 days (3 samples)	Low sensitivity and no specificity	No
Conventional culture	4-6 weeks	Needs time	Yes
Polymerase chain reaction	3-4 h	High cost	Yes
Immunodiagnosis	4-6 h	Lack of suitable antigens	No

TABLE 2
Main Protein Antigens Used in Diagnosis*

Antigen	Sensitivity	Specificity	Application	Study
ESAT6	27%		Latent TB	Silva et al[15]
CFP10	25%	97%	TB AFB-	Dillon et al[17]
16 kDa	57%		TB HIV+	Uma Devi et al[18]
30 kDa	61%	95%	Pulmonary TB	Sada et al[19]
38 kDa	68%	96%	Pulmonary TB	Espitia et al[20]
	63%	90%	TB HIV+	Ramalingam et al[21]
Mtb48	44.4%	93%	TB HIV+	Lodes et al[22]
TB16.3	66%		TB AFB-	Welding et al[23]
	88%		TB HIV+	Welding et al[23]
	98%		TB HIV-	Welding et al[23]
Mtb81	70%	99%	TB HIV+	Hendrickson et al[24]
U1 (21 kDa)	87.2%		TB HIV+	Mukherjee et al[25]

*AFB indicates acid-fast bacilli; TB, tuberculosis; HIV, human immunodeficiency virus.

TABLE 3
Lipid Antigens for Immunodiagnosis of Tuberculosis*

Antigen	Sensitivity	Specificity	Application	Study
Diacyl trehalose	81%-88%	96%-98%	Pulmonary and extrapulmonary TB	Escamilla et al[31]
Triacyl trehalose	91%-93%	96%-98%	Pulmonary TB	Escamilla et al[33]
Trehalose dimycolate	66%-74%	95%-99%	Pulmonary TB	López-Marín et al[25]
Sulfolipids (sulfolipid-1)	81%	77%	Pulmonary TB	Julián et al[34]

*TB indicates tuberculosis.

negative results for acid-fast bacilli and for diagnosis of extrapulmonary tuberculosis, for which sputum smears are not useful. Table 2 shows the main protein antigens used for diagnosis.[16-25]

To increase the sensitivity and specificity attainable with pure antigens, it has been proposed to use a mixture of different immunodominant antigens to cover the whole range of possible responses by all individuals in a population. A sensitivity of 90% was achieved with a battery of 9 antigens (ESAT6, 14 kDa, MPT63, 19 kDa, MPT64, MPT51, MTC28, 30 kDa, 38 kDa, and KatG).[14] One of the advantages of using protein antigens for immunodiagnosis is that they can be prepared using recombinant DNA technology. Such methods of preparation allow large-scale expression and purification with the corresponding reduction in cost of the test. One of the most innovative recombinant antigens tested for immunodiagnosis of tuberculosis is a recombinant multiepitope polyprotein (TbF6), which is expressed in *Escherichia coli* as a fusion protein, and which contains antigenic regions of Mtb8, 38 kDa, Mtb11, and Mtb48. With this recombinant antigen, a sensitivity of 94% was achieved.[25,26]

However, protein antigens are not the only candidates for use in immunodiagnosis. The cell wall of *M tuberculosis* is extremely rich in lipids, glycolipids, and polysaccharides, and a humoral immune response has been demonstrated against all these components during infection.[27,28]

Lipid antibodies: Of the lipids and glycolipids found in the cell wall of *M tuberculosis*, the trehalose lipids —diacyl trehalose, triacyl trehalose (TAT), trehalose monomycolate, trehalose dimycolate, and sulfolipid-1— are of particular note. These molecules have been shown to raise specific IgM, IgA, and IgG antibodies in both tuberculosis patients and murine models of infection.[29-32] It is therefore possible to design diagnostic methods that use purified lipid antigens adsorbed onto a solid matrix to screen for specific antibodies in serum samples from patients. Some of these antigens have proven useful; for example, higher sensitivity and specificity have been obtained with TAT than with protein antigens (Table 3).[31-33] An additional advantage of using lipid antigens is that the humoral response is more uniform than with protein antigens.

Polysaccharide Antibodies

Lipoarabinomannan (LAM) and its biosynthetic precursors, phosphatidyl-myo-inositol mannosides, are also abundant in the cell wall of *M tuberculosis*. Likewise, it has been shown that IgM and IgG antibodies are raised against these glycoconjugates during active infection.[34,35] It is particularly important to highlight that although LAM is widely distributed in different mycobacterial species, the type of capping differs for pathogenic and saprophytic species.[36] The slow-growing pathogenic species (*M tuberculosis, M bovis, M avium, M leprae,* etc) are capped with mannose residues (denoted manLAM), whereas fast-growing saprophytic species (*Mycobacterium fortuitum, Mycobacterium smegmatis,* etc) are either capped with arabinose residues (denoted araLAM) or are uncapped. It is therefore possible that the humoral response to LAM of the pathogenic mycobacteria may differ from that generated through contact with

(nonvirulent) saprophytic species. A sensitivity between 85% and 93% has been achieved with use of LAM.[37] Another polysaccharide to be tried as an antigen for immunodiagnosis is arabinomannan, although with little success because its structure hardly varies across mycobacterial species. Nevertheless, this polysaccharide has been shown to trigger a humoral response during infection (Table 4).[37,38] Unfortunately, the use of complex lipids and polysaccharides as antigens for serodiagnosis has the drawback that *M tuberculosis* has to be cultured and the corresponding antigens, some of which are present in very low concentrations in the cell wall, have to be purified.

Tests Based on Antigen Detection

An alternative immunological method for diagnosis of tuberculosis is detection of specific antigens for the pathogen in serum and other body fluids, particularly urine, through use of highly specific monoclonal antibodies. The most widely used antigen in immunoassays is once again LAM, followed by certain mycobacterial secretion proteins. As shown in Table 5, the immunological methods based on antigen capture can achieve higher sensitivity and specificity for diagnosis of tuberculosis.[39-43] A very important advantage of methods based on antigen detection is that they can be used for diagnosis of extrapulmonary tuberculosis.

Peptide Mimotopes of Nonprotein Antigens

Glycolipids and polysaccharides in the cell wall of *M tuberculosis* are excellent alternative antigens for serodiagnosis. However, they are limited in that they cannot be produced by recombinant techniques because they are secondary gene products and, furthermore, the metabolic pathways that synthesize many of them have yet to be elucidated. Nevertheless, small peptides of 7 to 15 amino acid residues have long been known to mimic the 3-dimensional structure of some epitopes of polysaccharides.[44,45] This means that such peptides, known as mimotopes because they mimic epitopes, can be used instead of complex lipids and polysaccharides, which cannot be synthesized chemically or generated by recombinant techniques. Currently, several systems are available commercially. These systems use a combinatorial peptide library expressed on filamentous phage displays to screen for sequences that are able to mimic the conformation of antigen determinants of complex molecules. Of the mimotopes that have been generated to mimic complex mycobacterial cell wall structures, the most noteworthy are those produced for antigen determinants of LAM for *M tuberculosis* and phenolic glycolipid-1 for *M leprae* (Table 4).[46,47] Investigators have yet to explore the potential of any of these mimotopes for serodiagnosis.

TABLE 4
Polysaccharides As Antigens for Immunodiagnosis of Tuberculosis*

	Antigen	
	LAM	AM
Sensitivity	85%-93%	56%
Specificity	89%-100%	
Application	Pulmonary TB	Pulmonary TB
Study	Chan et al[37]	Miller et al[38]

*TB indicates tuberculosis; LAM, lipoarabinomannan; AM, arabinomannan.

Other Tests

In many countries, particularly those where serodiagnosis is complicated by extensive cross reactions due to systematic vaccination programs or a high rate of infection with saprophytic mycobacteria found in the environment, methods based on the in vitro cell immune response to purified protein derivative (PPD) have started to be used. In these methods, the mononuclear cells of patients with a clinical diagnosis of tuberculosis are stimulated with PPD or with specific antigens (particularly ESAT6 and CFP10), and cell proliferation and production of interferon-γ are assessed.[48-51] The QuantiFERON-TB Test (Cellestis Ltd, Carnegie, Victoria, Australia), recently approved by the US Food and Drug Administration for the diagnosis of tuberculosis, is based on this system. A variation on quantification of in vitro production of interferon-γ is direct quantification of interferon-γ-producing cells with an enzyme-linked immunospot assay. Unfortunately, the need for in vitro culture of the cells of each patient pushes up the cost of diagnosis and limits its use in population studies.

Molecular Methods

Although immunological methods, particularly serologic ones, are reasonably simple to use in population studies, they cannot provide information on the species or strain responsible for infection. Therefore, the preferred approach to epidemiological surveillance has been genotyping by

TABLE 5
Mycobacterial Antigens Identified in Body Fluids*

Antigen	Sensitivity	Specificity	Application	Sample	Study
14 kDa	100%	100%	Meningeal TB	CSF	Sumi et al[39]
43 kDa	96.7%	100%	Pulmonary TB	Pleural fluid	Wadee et al[40]
	97.1%	100%	Peritoneal TB	Ascitic fluid	Wadee et al[40]
	96%	100%	Meningeal TB	CSF	Wadee et al[40]
LAM	74%	86.9%	Pulmonary TB	Urine	Tessema et al[41]
LAM	67%-88%	100%	Pulmonary TB	Serum	Sada et al[42]
LAM	94%	100%	Pulmonary TB	Sputum	Pereira et al[43]

*LAM indicates lipoarabinomannan; CSF, cerebrospinal fluid; TB, tuberculosis.

TABLE 6
Peptide Mimotopes of Mycobacterial Polysaccharides*

	Antigen	
	LAM	**PGL-1**
Species	*Mycobacterium tuberculosis*	*Mycobacterium leprae*
Mimotope	QEPLMGTVPIRAGGGS	WTLGPYV
Study	Gevorkian et al[47]	Youn et al[45]

*LAM indicates lipoarabinomannan; PGL-1, phenolic glycolipid-1.

isolating clinical cultures and applying molecular biology techniques. Recently, these techniques have started to be used diagnostically for direct identification of mycobacteria in body fluids of patients. However, *M tuberculosis* does not have a unique gene that allows the microorganism to be identified and diagnosed, and at least 3 genes or genomic regions have to be amplified to obtain a firm diagnosis.

Methods for Identifying Species

In the past, species corresponding to a clinical isolate were identified with a battery of sequential biochemical and physiological tests, and so an exact identification of the agent isolated from a patient's secretions often took a long time. In recent years, DNA hybridization and polymerase chain reaction (PCR) techniques, and the availability of complete genomes of many microorganisms of medical interest have revolutionized how we approach microbiological diagnosis and the epidemiology of infectious diseases.

Regions of difference. Modern molecular biology methods, particularly those based on PCR, can accurately identify a wide range of microorganisms, including mycobacteria, through the use of PCR primers for genus- and species-specific genomic sequences. The sequencing of the genome of *M tuberculosis*[52] and initial findings of comparative genomics[53-55] have allowed the identification of large regions of missing genetic material (deletions) that characterize the different mycobacterial species. Some of these regions of difference are restricted to species of the *M tuberculosis* complex and so can help distinguish between pathogenic and nonpathogenic mycobacteria (nontuberculous mycobacteria).

The method described by Huard et al[56] in 2003 used 6 PCR primer pairs for the Rv0577, Rv3349c, Rv1510, Rv1970, Rv3877/8, and Rv3120 loci. These primers generate amplicons of 400 to 1200 base pairs (Table 7), which allow the identification of *M tuberculosis, M africanum* subtype I, *M africanum* subtype II, *M bovis, M bovis*-BCG, *Mycobacterium caprae, M microti*, and *M canetti.* Furthermore, the mycobacterium corresponding to a clinical isolate can be classed as nontuberculous if amplification products from these genomic regions are not present.

Polymerase Chain Reaction–Restriction Fragment Length Polymorphism of hsp65

The *hsp65* gene has been used to distinguish between mycobacteria that do not belong to the *M tuberculosis* complex. This gene codes for a heat shock protein that is present in all mycobacteria.[57] In the method originally described by Telenti et al[58] in 1993, a 439-base pair region is amplified by PCR then digested with *Bst*EII and *Hae*III restriction enzymes. The restriction patterns of these enzymes for the amplified *hsp65* sequence can be visually identified by eye or a computer program can be used to automate the analysis. It has been possible to identify as many as 62 species of nontuberculous *Mycobacterium* species through use of other restriction enzymes such as *Sau*961 and *Cfo*I,[59] or *Ava*II, *Hph*I, and *Hpa*II.[60]

Methods for Genotyping Clinical Isolates of M tuberculosis

Genotyping of clinical isolates is done essentially with methods based on hybridization of DNA probes derived from repeat elements that characterize the *M tuberculosis* complex. The 2 most widely used techniques are Southern blot hybridization (restriction fragment length polymorphism [RFLP]-IS6110) and spoligotyping, although recently, the use of a variable number of tandem repeats (VNTR) has also been shown to be extremely useful.

RFLP-IS6110

Genotyping using RFLP takes advantage of discrete variations in the genetic material from different strains or clinical isolates. These variations can be detected by

TABLE 7
Polymerase Chain Reaction Primers for Identification of Clinically Important Mycobacteria

Target Region	Primers	Expected Product (Base Pairs)
Rv0577	5'-ATG CCC AAG AGA AGC GAA TAC AGG CAA-3' 5'-CTA TTG CTG CGG TGC GGG CTT CAA-3'	786
Rv3349c	5'-GCT GGG TGG GCC CTG GAA TAC GTG AAC TCT-3' 5'-AAC TGC TCA CCC TGG CCA CCA CCA TTG ACT-3'	943
Rv1510	5'-GTG CGC TCC ACC CAA ATA GTT GC-3' 5'-TGT CGA CCT GGG GCA CAA ATC AGT C-3'	1.033
Rv1970	5'-GCG CAG CTG CCG GAT GTC AAC-3' 5'-CGC CGG CAG CCT CAC GAA ATG-3'	1.116
Rv3877/8	5'-CGA CGG GTC TGA CGG CCA AAC TCA TC-3' 5'-CTT GCT CGG TGG CCG GTT TTT CAG C-3'	999
Rv3120	5'-GTC GGC GAT AGA CCA TGA GTC CGT CTC CAT-3' 5'-GCG AAA AGT GGG CGG ATG CCA GAA TAG T-3'	404

digesting the DNA with frequent-cutter restriction enzymes, which generate digestion patterns that can allow the different strains to be distinguished.[61] The most commonly used restriction enzymes (*Bst*EII and *Bcl*I) generate multiple bands, but this large number of bands hinders analysis so investigators increasingly use radiolabeled probes derived from repeat elements—insertion sequences—that vary in terms of the number of copies and their distribution in the genomes of the mycobacteria. The technique requires the following steps: DNA extraction from strains isolated by culturing; digestion of the DNA with a restriction enzyme that cuts the insertion element (generally *Pvu*II for cutting IS6110); resolution of the digested DNA on agarose or polyacrylamide gel and transfer to nitrocellulose membranes; and hybridization with a radiolabeled probe for IS6110, although currently a nonradioactive label with biotin or digoxigenin can be used. Given that *M tuberculosis* contains 8 to 20 copies of IS6110 (according to the strain), this method can detect 16 to 40 bands, which is enough to differentiate and classify clinical isolates.[62]

Another of the insertion sequences used for distinguishing between mycobacterial strains is IS1081 (1324 base pairs), which is found in strains of the *M tuberculosis* complex but, due to the low number of copies, is of limited use in epidemiological studies. Techniques based on IS1081 also have the drawback of not being able to differentiate between *M bovis*-BCG and other members of the *M tuberculosis* complex.[63]

Spoligotyping

Spoligotyping takes advantage of the extensive polymorphism of the DR locus of *M tuberculosis*.[64] This locus contains direct repeats of 36 base pairs, interspaced by nonrepetitive sequences of 34 to 41 base pairs. Strains vary according to the number of direct repeats as well as the presence or absence of some spacers. With spoligotyping, conserved sequences of direct repeats are used as a target for PCR amplification, and the extensive variability in the spacer regions is used to obtain different hybridization patterns of the amplified product for comparison with patterns produced by synthetic spacer oligonucleotides covalently immobilized on a nitrocellulose membrane. The method described by Kamerbeek et al[65] in 1997 makes use of 43 spacers which not only can be used to type strains of *M tuberculosis* but also to distinguish them from *M bovis* and *M bovis*-BCG.

In 2002, van der Zanden et al[66] introduced 51 new spacers thereby considerably increasing the capacity of this technique for genotyping.

VNTR

Like the minisatellites described in eukaryotic genomes, 41 regions of 40 to 100 base pairs of repeat sequences arranged in tandem have been found in *M tuberculosis*. These sequences are known as mycobacterial interspersed repetitive units (MIRU). Of these, 12 loci are sufficiently polymorphic in terms of number of copies in clinical isolates of *M tuberculosis* and have started to be used successfully in epidemiological studies.[67] In addition, the use of MIRU-VNTR for genotyping clinical isolates has the advantage that the process can be automated.

Conclusions

Molecular strategies are clearly an essential part of the microbiological studies of tuberculosis because they can accurately determine the species to which a given clinical isolate belongs in a matter of hours. Furthermore, acceptable epidemiological monitoring is possible with spoligotyping or VNTR. Unfortunately, although molecular techniques are sensitive enough to detect DNA from just 2 mycobacteria, their application in tuberculosis diagnosis has so far not been as successful as hoped, mainly because of the presence of PCR inhibitors in clinical samples. However, once the etiologic agent has been isolated by culturing clinical samples in conventional media, the use of PCR can reduce the time needed to identify the species to a few hours. Another reason why samples will continue to be cultured is that no other technique can be used so successfully in drug sensitivity studies, even though several molecular methods have been described for the analysis of mycobacterial genes associated with drug resistance.[68]

The characteristics that make serologic methods so attractive (simplicity, low cost, etc) will allow these techniques to replace reliance on sputum smears for diagnosis, once the problem of availability of specific antigens has been overcome. Population studies can then be designed to investigate the prevalence of tuberculosis.

The predictive value of the different methods has not yet been fully determined, particularly in the case of methods still in development. Sputum smears, for example, have a low sensitivity (10%), and so a negative value does not rule out the presence of the bacillus, whereas a positive result is reliable evidence of the presence of *M tuberculosis* in a patient with clinical signs and symptoms of the disease. In turn, traditional culturing has a higher predictive value (95%) and, along with biochemical tests, is the benchmark technique for identifying *M tuberculosis*, but of course the time required to reach an accurate diagnosis is extremely long.[69] The sensitivity and specificity of innovative immunological methods for antibody detection are generally low, and although methods involving capture and detection of mycobacterial antigens, particularly glycolipids, appear promising, they are still not used systematically, mainly because specific monoclonal antibodies are lacking and/or are not commercially available. Finally, of the molecular methods under evaluation, PCR appears the best option for directly identifying *M tuberculosis* although it can only detect the presence of genetic material and does not provide information on the viability of the bacillus. We are thus drawn to conclude that we still lack a rapid diagnostic strategy that meets all the requirements for implementation on a sufficiently large scale to deal with the spread of the disease. Clinical judgment and laboratory tests are therefore still required, and the search for new strategies should continue.

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