

The Tuberculin Skin Test: Time for a Change?

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Tuberculosis (TB) continues to cause high morbidity and mortality rates throughout the world.¹ Early diagnosis and appropriate treatment are essential to control the spread of this disease. The microbiological methods of reference in TB diagnosis continue to be microscopic study, the culture and isolation of *Mycobacterium tuberculosis*, and the detection of its nucleic acids. Nevertheless, it is well known that the current techniques are inadequate and that infected persons are a potential hazard of new TB cases. There are an estimated 2000 million infected persons in the world. The study of these persons allows measures to be taken to prevent them from developing the disease and thus help break the chain of transmission of the microorganism.

The study of the delayed, hypersensitivity response to certain antigen components specific to *M tuberculosis* determines whether an individual has been infected with the bacillus. This is the principle of tuberculin, which is obtained from sterilized, concentrated *M tuberculosis* culture filtrate and currently consists of a purified protein derivative (PPD). Tuberculin has been used in the diagnosis of TB for 100 years, its main drawback being that most of the proteins present in the PPD are not specific to *M tuberculosis* but are common to other mycobacteria. The test is therefore less specific as persons sensitized from prior exposure to other mycobacteria or who have received an antituberculin vaccination (attenuated *Mycobacterium bovis* strains) will also have an immunological response to the PPD. There are several other disadvantages to its use: *a*) poor sensitivity in immunosuppressed persons with deficient cellular immunity, *b*) management difficulties with young children, *c*) errors in the administration of tuberculin, *d*) subjective interpretation of the results, and *e*) an additional visit needed for reading the results. The fact that the patient has to return 2 or 3 days later for the results of the test to be read causes worry and anxiety

and additional loss of working hours, not to mention the loss of the considerable number of patients who do not return.²

The natural progression of the disease is the following. The main route of infection is the arrival of *M tuberculosis* to the lung alveoli, where it is absorbed by the alveolar macrophages. These macrophages release cytokines which attract neutrophils, lymphocytes, and more macrophages to absorb the extracellular bacilli and to produce an inflammatory focus. With the mediation of interleukin-12, secreted by the macrophages, the specific T CD4 lymphocytes differentiate into helper T1 (T_H1) cells. T_H1 cells secrete tumor necrosis factor alpha (TNF- α) to attract more macrophages and interferon-gamma (IFN- γ) to activate the infected ones. Gammadelta T lymphocytes (T $\gamma\delta$) also secrete IFN- γ to activate macrophages and interleukin-12 to promote proliferation of T_H1 cells. Moreover, macrophages synthesize interleukin-12, which stimulates cytolytic cells. Those cells also synthesize IFN- γ , which is the key effector cytokine in the control of mycobacterial infection through its activation of macrophages and in the development of protective immunity against *M tuberculosis*.³ An immunodiagnostic method based on the *in vitro* quantification of the cellular immune response could thus be an alternative to tuberculin testing in the identification of TB infection.

To that effect, several methods of quantifying cellular immune response have been developed using various mycobacterial antigens to stimulate the sensitized T cells and to detect the release of IFN- γ *in vitro*. The approach consists of stimulating the lymphocytes *in vitro* with mycobacterial antigens and then detecting the IFN- γ produced using immunological techniques.⁴ The success of this method largely depends on the antigens used for stimulation. Initially, PPD was used. PPD is a poorly defined complex mixture of antigens, comprised mainly of proteins in varying stages of denaturation and several studies showed problems of specificity.^{5,6} However, 2 antigens secreted by the *M tuberculosis* complex that are absent from the TB vaccine and from other environmental mycobacteria—namely the early secretory antigen target-6 (ESAT-6) and the culture filtrate protein 10 (CFP-10)—appear more successful in the detection of *M tuberculosis* infection.⁷⁻⁹ Recent studies have shown their utility as indicators of risk of

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progression to TB in exposed patients.¹⁰ Nevertheless, positive reactions have been described in individuals in frequent contact with certain environmental mycobacteria, in patients infected with *Mycobacterium leprae*, and even in vaccinated persons.¹¹

These limited and at the same time encouraging results justify the need to standardize the antigens used, to develop more sensitive and specific techniques, and to carry out rigorous assessments to determine actual utility. There are currently 2 commercialized methods available to diagnose TB infection: Quantiferon Gold (Cellestis, Australia) and T-SPOT-TB (Oxford Immunotec, United Kingdom). Both stimulate specific lymphocytes by means of ESAT-6 and CFP-10 and subsequently detect IFN- γ production. However, differences between the methods must be taken into account. Quantiferon Gold stimulates lymphocytes present in whole-blood samples and quantifies IFN- γ production by enzyme-linked immunosorbent assay whereas T-SPOT-TB requires separation of mononuclear cells before stimulation and determines the presence of IFN- γ by enzyme-linked immunosorbent spot assay.

Our research team is investigating the scope of these new techniques. We do not yet have the sample size we would like to acquire before publishing results. Nevertheless, we believe our preliminary findings have shown that both methods produce similar results and are possible alternatives to tuberculin testing. Assessment of these techniques is complicated by the lack of a gold standard to use for comparison, tuberculin not being completely specific. Studies of contacts have shown these techniques to correlate with the degree of exposure to *M tuberculosis* better than tuberculin testing does; moreover, the TB vaccine does not affect results.^{8,12} According to our preliminary assessments of sensitivity and specificity, and using the latest versions of the 2 assays, our impression is that they will be appropriate both for studies of contacts and screening as well as for more specific studies on immunodepressed individuals, pediatric populations, and TB patients.

The possible use of these in vitro techniques as standardized laboratory tests would provide certain advantages over tuberculin testing: results interpretation subjectivity is avoided; the assay can be repeated immediately if necessary; results are obtained quickly; a further visit to read the results is not necessary; loss of persons who do not return for reading of results is avoided; the test is easily standardized and applied in the laboratory, and controls can be included in all series to detect responses to environmental mycobacteria, responses due to TB vaccination, or patients with anergic responses. Moreover, the assays are performed in the laboratory where patients' privacy is maintained rather than in a visible place as occurs with tuberculin testing. One of the main drawbacks could be the cost of the assays, currently more expensive than tuberculin tests. However, preliminary studies have shown that, in

terms of overall cost-effectiveness, health systems would benefit from using the new techniques instead of tuberculin skin tests (chemoprophylaxis would no longer be needed, fewer working hours would be lost, etc), even without taking into account that the cost of the new assays will decrease across the board once use increases.

In summary, detection of TB infection by tuberculin is less than perfect and efforts should thus be made to standardize in vitro techniques based on the immune response of the host to the pathogen. The techniques currently being investigated appear to be possible alternatives to tuberculin. The tests distinguish persons sensitized by *M tuberculosis* from those who have received TB vaccine and from those exposed to other mycobacteria. In our experience the tests are easy for a trained laboratory staff to use and they simplify the study of TB contacts.

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