

# APŽVALGINIS STRAIPSNIS

## Research for practice: a new *in vitro* test for identification of tuberculosis infection

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**Key words:** tuberculosis; tuberculin skin test; interferon-gamma; ELISPOT; T-SPOT TB.

**Summary.** Tuberculosis is one of the biggest global health problems. One-third of the world's population (2 billion) is latently infected with tuberculosis. The tuberculin skin test is commonly used to diagnose tuberculosis infection. This test has poor specificity and sensitivity, cross-reactivity with bacille Calmette-Guérin vaccination and many environmental mycobacteria, and poor sensitivity (only 75–90% in active tuberculosis). *Mycobacterium tuberculosis* activates a strong T cell-mediated immune response. That is why, a better marker for tuberculosis infection could be the presence of mycobacteria specific interferon- $\gamma$ -secreting T cells. These cells can be identified in blood or any other sample, which contains T cells. The test specificity is 99.9% (in low-risk control groups), and the sensitivity is 97.2% (in subjects with culture-confirmed active disease).

New *in vitro* diagnostic test of tuberculosis, based on tuberculosis-induced immunological mechanisms, seems to be more specific and useful as previous methods.

### Introduction

Tuberculosis (TB) is one of the most serious global health problems today. *Mycobacterium tuberculosis* causes two million deaths every year (1, 2). Every year 8 million of new cases of TB are diagnosed worldwide (1, 2). One-third of the world's population (2 billion) is latently infected with TB. Infection rates continue to raise leading to TB being described as a clinical time bomb (1–4). Up to 10% of those who are latently infected go on to get active disease. If to control TB, it is necessary to eliminate latent TB infection. This means that infected people should be identified before they get active disease and transmit TB to others.

### General characteristics of *Mycobacterium tuberculosis*

Mycobacteria are gram-positive bacteria belonging to the genus *Actinomyces* (1, 3).

*Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, and *Mycobacterium microti* are members of TB complex and can cause tuberculosis (3, 5).

Some of the main characteristics of *M. tuberculosis*

(1, 3, 5):

- obligate aerobes, which thrive in high oxygen tensions, including the lungs, especially the upper lobes;
- prefer moderate temperature for growing (25–41°C);
- do not form spores;
- intracellular pathogens that can infect macrophages;
- grow very slowly (generation time is 12–20 hours);
- acid-fast bacilli or acid-alcohol-fast.

The course of the infection, clinical signs, and likelihood of developing active disease depend on virulence of the *M. tuberculosis* strain and the immune system of the infected subject.

### Immunopathology of tuberculosis

Only 10% of people infected with *M. tuberculosis* develop active disease (1, 2). The risk of developing active disease increases in immunosuppressed subjects (1).

Information concerning the early events following the inhalation of *M. tuberculosis* is limited. Tiny droplets containing *M. tuberculosis* are breathed in and

are directed to the terminal bronchi or alveoli. These mycobacteria are ingested by alveolar macrophages, which are then killed as mycobacteria multiply producing local inflammation. Other phagocytic cells and neutrophils are also involved (1, 6). Granuloma may be formed. Some mycobacteria are transported to hilum of the lungs where they can form the primary complex.

Th1 cells surround the granuloma of the primary complex and secrete cytokines, especially interferon- $\gamma$  (INF- $\gamma$ ), which will activate macrophages. Macrophages become more cytotoxic. They consume oxygen so that mycobacteria in the center of granuloma begin to die leading to cessation (1). Many mycobacteria die leading to calcification. Some of mycobacteria can be persistent in the human body for many years (1). They can multiply leading to the reactivation of TB. This is postprimary TB, which can be seen as tuberculomas and formed pulmonary cavities (1). There are some immunological differences between primary and postprimary TB.

Th1 cells are more important in protective responses in primary TB (Fig.) (6). Th2 cells play a role in postprimary lesions and are responsible for extensive necrosis. This can be result of faulty regulation of the activity of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). When a protective granuloma develops, it is destroyed by this faulty immunological response and process continues (1, 5).

CD4<sup>+</sup> cells are largely responsible for TB immunity, but they are supported by other T cells such as CD8<sup>+</sup>,  $\gamma$ ,  $\beta$  TCR<sup>+</sup> cells, CD1<sup>+</sup> cells (1, 5–8). T cells help macrophages to control *M. tuberculosis*, but the exact mechanism is poorly understood. Cytokines that take part in the immune response to *M. tuberculosis* are TNF- $\alpha$ , IL-12, INF- $\gamma$  (from CD4<sup>+</sup> and CD8<sup>+</sup>).

During primary infection, macrophages control the growth and replication of *M. tuberculosis*. Immunosuppression allows the infection to spread often producing a different clinical picture. The interaction be-

tween T cells and infected macrophages determines level of infection (5).

### Diagnosis of tuberculosis

TB is diagnosed by clinical examination, the patient's history, and *in vitro* diagnostic tests.

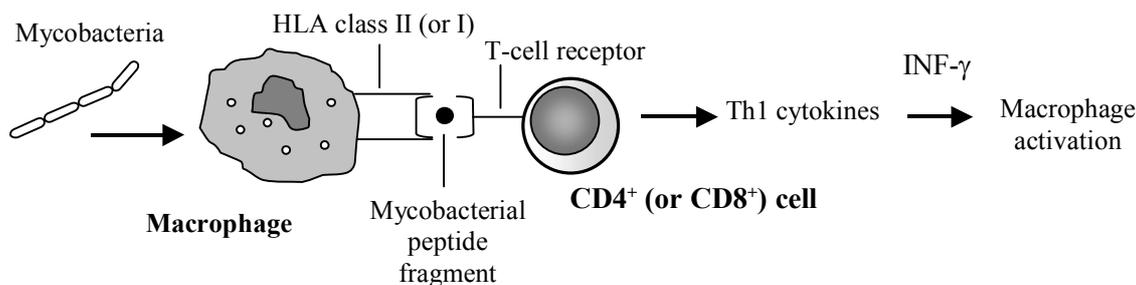
For many years, the tuberculin skin test (TST) has been used to diagnose latent and active TB. However, it has poor specificity and sensitivity (only 75–90% in culture confirmed active disease) (1, 2). It produces many false-negative and false-positive results (2, 9–11).

Some of the disadvantages of the TST include (1, 2, 8, 11):

- antigenic cross-reactivity with bacille Calmette-Guérin (BCG) vaccination producing a false-positive reaction;
- antigenic cross-reactivity with environmental mycobacteria producing a false-positive reaction;
- operator variability in giving and reading the test;
- negative TST in patients with meningitis or viral infection including varicella, *etc*;
- negative TST after vaccination with a live vaccine (*e.g.* the first three weeks following vaccination against varicella, rubella, *etc*);
- false-positive reaction in patients who received BCG vaccination (according to the different data 3–5 years);
- negative or weak TST reaction in active TB disease (sensitivity is only 75–90% – anergy);
- poor response in immunosuppressed patients;
- negative TST in newborns (the first 3–4 weeks);
- booster effect if TST is repeated a number of times;
- the test requires two patient visits, one to give the test and one to have it read;
- about one-third of those who are given the TST never return to have the result read.

Consequences of the inaccuracy of TST (2, 9):

1. If TST gives a false-negative result, infected sub-



**Fig. Specific immune response to mycobacteria**  
HLA – human leukocyte antigen; INF- $\gamma$  – interferon- $\gamma$ .

jects will be missed. They may develop active disease or their condition will become worse, causing a large burden on the health care system. These subjects can infect other people leading to the spread of the disease.

2. If TST shows a false-positive reaction, the subject does not have TB but will be given unnecessary treatment, which may cause toxicity, particularly in the liver.

Tests for the diagnosis of active disease:

The gold standard method for identification of TB involves collection of sputum or other liquid, which is then cultured (1–5).

Polymerase chain reaction is a newer test that is very specific but may not always be sensitive as it is not always possible to obtain mycobacteria in the specimen (1, 2).

Radiology is one of the most used for diagnosis of tuberculosis, but not all forms of TB can be identified, particularly nonpulmonary TB (1).

Newer tests which use T cells, *in vitro*, with very specific antigens only found in TB (ESAT 6 and CFP 10) offer much promise (2, 10–13).

In 2001, Ewer and colleagues examined 1128 students who were in contact with sputum smear-positive TB patients (10). They performed TST and ELISPOT (immunological assay based on enzyme-linked immunosorbent for determining the amount of activated an-

tigen-specific cytotoxic INF- $\gamma$  in blood). The degree of exposure to the index case was compared to the positivity of two tests. Although, agreement between the tests was high (89% concordance,  $k=0.72$ ,  $P<0.0001$ ), ELISPOT correlated significantly more strongly with *M. tuberculosis* than TST did. TST was more likely to be positive in BCG-vaccinated than in non-vaccinated students (10).

Other study was carried out in 2004. Meier and colleagues performed a study on patients with confirmed or suspected TB. The results of patient blood INF- $\gamma$  test (T-SPOT TB) were compared with results of conventional diagnostic tools. The sensitivity of T-SPOT TB test was 97% (14).

In 2001, Liebeschuetz and colleagues studied children with suspected TB disease. A large number of TST-negative children with TB were positive in ELISPOT indicating that T-SPOT TB is more sensitive. This discordance became more marked when additional confounding factors such as being infected with HIV, being malnourished, or being less than 3-year old were present (15).

This test is routinely used in more than 20 countries. There are ongoing 16 clinical trials on T-SPOT TB. More than 4000 subjects are participating in these trials (2).

New *in vitro* diagnostic test of TB, according to the TB-induced immunological mechanisms, seems to be more specific and useful than previous methods.

## Mokslas – praktikai. Tuberkuliozės infekcijos diagnozavimas nauju *in vitro* testu

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**Raktažodžiai:** tuberkuliozė, tuberkulino mėginys,  $\gamma$ -interferonas, ELISPOT, T-SPOT TB.

**Santrauka.** Tuberkuliozė išlieka viena didžiausių sveikatos problemų visame pasaulyje. Trečdalis pasaulio populiacijos (2 milijardai) yra užsikrėtę tuberkuliozės mikobakterijomis. Dažniausiai naudojamas tuberkuliozės infekcijos diagnostikos metodas yra tuberkulino mėginys, kurio jautrumas ir specifiskumas tik 75–90 proc. aktyviosios tuberkuliozės atveju. Atsižvelgiant į tai, kad didžiausią įtaką tuberkuliozės infekcijos plitimui organizme turi ląstelinis imunitetas, sukurti nauji tuberkuliozės infekcijos diagnostikos metodai. Tyrimo metu vertinamas  $\gamma$ -interferono išskyrimas iš tiriamojo T limfocitų. Tiriamas kraujas ar bet kuri kita tiriamoji medžiaga, kurios sudėtyje yra T limfocitų. Testo specifiskumas – 99,9 proc. (mažos rizikos grupėse), jautrumas – 97,2 proc. (bakteriologiškai patvirtintos tuberkuliozės atveju).

Naujas *in vitro* tuberkuliozės diagnostikos metodas, pagrįstas tuberkuliozės infekcijos sukeliama imunologiniais pokyčiais, yra perspektyvesnis už ankstesnius.

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## References

1. Pratt JR, Grange JM, Williams VG. Tuberculosis. A foundation for nursing and healthcare practice. Immunity and immunopathology in tuberculosis. Oxford: Oxford University Press; 2005. p. 61-79.
2. Oxford Immunotec. T SPOT Presentation 2006. Available from: URL: <http://www.oxfordimmunotec.com>
3. Microbiology Library: Mycobacterium tuberculosis 2002. Available from: URL: <http://www-micro.msb.le.ac.uk/video/Mtuberculosis.html>
4. Barnes PF. Diagnosing latent tuberculosis infection: the 100-year upgrade. Am J Respir Crit Care Med 2001;163:807-8.
5. Lewinsohn DA, Gennaro ML, Scholvinck L, Lewinsohn DM. Tuberculosis immunology in children: diagnostic and therapeutic challenges and opportunities. Int J Tuberc Lung Dis 2005;815:658-74.
6. Chapel H, Haeney M, Misbah S, Snowden N. Mycobacterial infection. In: Essentials of clinical immunology, 4th ed. Oxford: Blackwell Science Ltd; 1999. p. 42-5.
7. Richeldi L, Ewer K, Losi M, Bergamini BM, Roversi P, Deeks J, et al. T-cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. Am J Respir Crit Care Med 2004;170:288-95.
8. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet 2000;356:1099-104.
9. Menzies D. Interpretation of repeated tuberculin tests, boosting, conversion, and reversion. Am J Respir Crit Care Med 1999;159:15-21.
10. Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Anderse P, et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacteria tuberculosis* infection in a school tuberculosis outbreak. Lancet 2003;361:1168-73.
11. Zellweger JP, Zellweger A, Ansermet S, de Senarclens B, Wrighton-Smith P. Contact tracing using a new T-cell-based test: better correlation with tuberculosis exposure than tuberculin skin test. Int J Tuberc Lung Dis 2005;9(11):1242-7.
12. Lalvani A, Pathan AA, McShane H, Wilkinson RJ, Conlon CP, Pasvol G, et al. Rapid detection of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. Am J Respir Crit Care Med 2001;163:824-8.
13. Lalvani A, Pathan AA, Durkan H, Wilkinson KA, Whelan A, Deeks JJ, et al. Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen specific T cells. Lancet 2001;357:2017-21.
14. Meier T, Eulenbruch HP, Wrington-Smith P, Enders G, Regnath T. Sensitivity of new commercial enzyme-linked immunospot assay (T SPOT TB) for diagnostics of tuberculosis in clinical practice. Eur J Clin Microbiol Infect Dis 2005; 24:529-36.
15. Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. Lancet 2004;361:364-96.

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