ELISPOT

Spotting latent infection: the path to better tuberculosis control

A Lalvani

The new ELISPOT assay will help control tuberculosis

Tuberculosis (TB) control is based on prevention as well as prompt diagnosis and treatment of active TB. Since the latter is usually accomplished quite effectively in developed countries, and since BCG vaccination is of limited effectiveness, better TB control will require improved diagnosis and preventative treatment of latent tuberculosis infection (LTBI).[1–3] The reservoir of latently infected individuals is much larger than the number of active TB cases, and includes recently infected contacts of pulmonary TB cases and immigrants from high prevalence regions who acquired infection in their country of origin. This latter group is becoming increasingly important because over half the burden of TB in many low prevalence countries is carried by immigrants,[3–5] and because several higher prevalence countries will soon join the European Union.

Prophylactic treatment of LTBI is highly effective in preventing the subsequent development of active TB;[1] the difficulty lies in identifying who is harbouring latent bacilli. TB control programmes rely exclusively on the century old tuberculin skin test (TST) for diagnosing LTBI in asymptomatic individuals with known or suspected TB exposure.[1 3] The success or otherwise of TB control and elimination in the developed world thus hinges on the oldest diagnostic test in medicine, and the multiple limitations of the TST constitute a major roadblock to better TB control.

The main drawback of the TST is its poor specificity because of false positive results in BCG vaccinated individuals caused by antigenic cross reactivity of purified protein derivative (PPD) with BCG;[6] this confounding effect persists for as long as 15 years after vaccination.[7] This is a widespread problem as most of the world's population is BCG vaccinated and, even in low prevalence countries that have ceased BCG vaccination, most TB cases and their contacts are BCG vaccinated immigrants. The problem is so significant that the British Thoracic Society Code of Practice for Control and Prevention of TB no longer recommends performing the TST on BCG vaccinated adults with recent TB exposure.[8]

The recent identification of genes present in *Mycobacterium tuberculosis* but absent from BCG raises the possibility of developing a more specific diagnostic test.[9 10] Detecting an immune response to one of these gene products could, in theory, indicate *M tuberculosis* infection as distinct from BCG vaccination.[11] However, humoral immune responses in LTBI are generally weak, and this has proved to be an insurmountable barrier to the development of a useful serological test.[11] Individuals with LTBI (and most patients with active TB) do, however, mount a strong cellular immune response to *M tuberculosis*. Fortunately, two of the proteins that are absent from BCG are major targets of the T cell response to *M tuberculosis*—early secretory antigenic target 6 (ESAT-6)[12] and culture filtrate protein 10 (CFP10).[13]

ENZYME LINKED IMMUNOSPOT (ELISPOT)

Measurement of T cell responses has traditionally been confined to the research laboratory as it required specialised sterile tissue culture facilities, technical expertise, and radioisotopes. However, the most sensitive assay for detecting antigen specific T cells was recently modified to enable rapid and convenient detection of T cells directly from a blood sample.[14] The rapid ex vivo enzyme linked immunospot (ELISPOT) assay counts individual antigen specific T cells. T cells from individuals infected with *M tuberculosis* become sensitised to ESAT-6 or CFP10 in vivo; when the T cells re-encounter these antigens ex vivo in the overnight ELISPOT assay they release the cytokine interferon-γ.[15] By the next morning each such T cell gives rise to a dark spot which is the ''footprint'' of an individual *M tuberculosis* specific T cell. The read out is thus the number of spots, which are counted using a magnifying lens or automated reader. The principle that underpins ELISPOT is that a highly sensitive T cell assay using highly specific *M tuberculosis* antigens should result in a test with high diagnostic sensitivity and specificity. So what happens in the clinic?

Clinical studies

ELISPOT was first validated and compared with TST in patients with culture confirmed active TB and control patients with non-tuberculous illnesses; its sensitivity was 96%, significantly higher than the 69% for TST.[16] Importantly, non-tuberculous illnesses did not cause false positive results. Unlike TST, ELISPOT is not susceptible to false negative results in patients with disseminated TB and it maintains its high sensitivity it HIV infected TB patients.[17] ELISPOT may thus prove clinically useful in the diagnostic assessment of patients with suspected active TB in low prevalence regions; in particular, its high sensitivity could help clinicians to rule out a diagnosis of TB.[18]

Demonstrating superiority of a new test for LTBI is more difficult than for active TB because there is no gold standard reference test. Thus, it is not possible to measure directly the sensitivity and specificity of a new test for LTBI. However, as airborne transmission of *M tuberculosis* is promoted by increasing duration and proximity of contact with an infectious case, a key determinant of infection is the amount of time spent sharing room air with the source case. If ELISPOT is indeed a more sensitive and specific test, it should therefore correlate more closely with the level of exposure to *M tuberculosis* than the TST, and should be independent of BCG vaccination status.

A community study of 50 recent TB contacts at risk of LTBI found that ELISPOT correlated with the extent of recent exposure to cases of pulmonary TB, as judged by exposure history, whereas unexposed people were uniformly ELISPOT negative.[19] Unlike TST, ELISPOT was not confounded by BCG vaccination status.[19] However, proving a statistically significant better correlation with exposure is a major challenge, as it would require simultaneous screening by ELISPOT and TST of large numbers of people with a wide range of precisely quantified exposure to *M tuberculosis*. In 2001 the UK suffered its largest outbreak of TB since the Second World War. It occurred in a secondary school and resulted from a single infectious source case with several hundred contacts; school timetables permitted precise quantification of the amount of time each child spent sharing room air with the source case. 535 students were tested by ELISPOT and TST in a blinded, prospective study, and correlation of each test with degree of exposure to the source case and BCG vaccination status was compared. Although agreement bet-

ween the tests was high (89% concordance), ELISPOT correlated significantly more closely with *M tuberculosis* exposure than TST, based on predefined measures of proximity and duration of exposure to the source case.[20] TST was significantly more likely to be positive in BCG vaccinated students whereas ELISPOT was independent of BCG vaccination. Thus, although direct quantification of sensitivity and specificity of ELISPOT or TST for LTBI is not possible in the absence of a gold standard, the unique circumstances of this outbreak made it possible to rank the tests according to their diagnostic accuracy.[20]

What more do we need to know?

Three thousand individuals in seven countries have been tested by ELISPOT to date; the results from the first 1000 have already been published[16 17 19–21] and indicate that ELISPOT is a more accurate marker of LTBI than TST. What more do we need to know before we can use ELISPOT to guide the management of LTBI? Notwithstanding the numerous limitations of the TST, several decades of long term follow up studies have shown that a strongly positive TST in exposed asymptomatic individuals has some predictive value for subsequent development of active TB. Thus, the cross sectional data indicating that ELISPOT is more accurate than TST should be supplemented by some longitudinal data to confirm that exposed individuals with a positive ELISPOT result really are at risk of subsequent active TB. Despite the long incubation period of TB, clinical outcome data of this sort are already beginning to emerge from several ongoing longitudinal studies around the world. In addition, we need to know how reliably ELISPOT performs in high throughput routine hospital laboratories. ELISPOT only requires a centrifuge, incubator and microscope and has been successfully transferred to several rudimentary laboratories in resource poor settings; thus, we already know that it is simple and robust. Nonetheless, commercial development of the assay through to regulatory approval, which is already underway, is making ELISPOT even faster and better suited to high throughput laboratories.

IMPACT OF ELISPOT

Once ELISPOT enters routine practice, how will it impact on TB control? We can try to predict this on the basis of its three key attributes:

- high specificity;
- high sensitivity;
- it is an ex vivo blood test rather than an in vivo skin test.

High specificity

The improved specificity of ELISPOT will mean that, in BCG vaccinated populations, targeted screening and treatment for LTBI could be performed more widely and vigorously without anxiety about false positive results due to prior BCG vaccination. It would also avoid unnecessary chemoprophylaxis and its attendant toxicity. This ability to screen out false positive TST results will become increasingly important as the prevalence of LTBI falls in low prevalence countries. This is likely to be an enabling step for control programmes that aim to eliminate TB, such as those of the United States Centers for Disease Control[1 2] and the European Working Group on Control and Elimination of TB.[3]

High sensitivity

Although the sensitivity of TST for LTBI cannot be directly quantified, we know that false negative results are common in at least two important groups: HIV infected individuals and those on immunosuppressive drugs.[1 6] This is a significant problem because it is precisely these people who, once infected, are at highest risk of progression to active TB.[1] Comparative studies to date indicate that ELISPOT has a higher sensitivity than TST in people with HIV induced[17] or iatrogenic immunosuppression (L Richeldi and A Lalvani, unpublished observations). False negative TST results also occur in contacts who already have active TB at the time of screening. The higher sensitivity of ELISPOT for active TB will help to minimise this problem.[16] Thus, the improved sensitivity of ELISPOT over TST in these groups should help to reduce the burden of active TB.

Blood test rather than skin test

The fact that ELISPOT is a blood test will have three major consequences: (1) the problem of people not returning to have their skin tests read will be circumvented and this should increase the yield of contact investigations and screening for LTBI; (2) repeated testing of high risk individuals such as healthcare workers would not be confounded by the booster phenomenon where repeated skin testing eventually induces false positive TST results[1 6]; and (3) test results will be issued by hospital laboratories instead of being read by contact clinic nurses, thus increasing the workload in laboratories while decreasing the workload in contact clinics. The operational consequences of this are hard to predict, but it could allow overburdened contact clinic personnel to focus on contact tracing and adherence with preventative treatment rather than administering and reading TST results.

Since the TST is cheap, the introduction of ELISPOT would initially increase the cost of TB control. However, the cost savings that would follow from avoiding unnecessary chemoprophylaxis and from reducing the number of cases of active TB could make ELISPOT very cost effective in the long term. The World Health Organisation is undertaking a quantitative cost-benefit health economic analysis of the recent use of ELISPOT to prevent a potential outbreak of multidrug resistant TB in northern Italy.

For high burden countries, improving prompt diagnosis and treatment of active disease remain the immediate priorities. However, better diagnosis of TB infection by ELISPOT could help TB control in high burden countries in three ways: (1) by improving diagnosis of asymptomatic infection (and active TB) in children; (2) by improving diagnosis in HIV infected individuals;[17] and (3) by enhancing epidemiological surveys to assess the effect of TB control measures.[21] Thus, although the greatest impact of ELISPOT will initially be on TB control in the developed world, it is likely that countries with a high burden of TB and HIV will also stand to benefit from this new approach to spotting TB infection.

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