Counting Antigen-Specific T Cells: A New Approach for Monitoring Response to Tuberculosis Treatment?

Ajit Lalvani
Nuffield Department of Clinical Medicine, University of Oxford, United Kingdom

(See the brief report by Carrara et al. on pages 754–6)

A new approach to diagnosis of *Mycobacterium tuberculosis* infection based on rapid detection of *M. tuberculosis*–specific T cells was recently developed [1]. The ex vivo enzyme-linked immunospot (ELISPOT) assay enumerates T cells specific for 2 small, intriguing antigens that are secreted by *M. tuberculosis* but are absent from all strains of *Mycobacterium bovis* bacille Calmette-Guérin (BCG): early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP10). T cells from *M. tuberculosis*–infected individuals become sensitized to ESAT-6 or CFP10 in vivo; when the T cells reencounter these antigens ex vivo in the overnight ELISPOT assay, they release a cytokine, IFN-γ [2]. 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, and the readout is the number of spots. With use of peptides spanning the length of these antigens, the ELISPOT assay has a sensitivity of 96% for patients with culture-confirmed tuberculosis. This is significantly higher than the sensitivity of the tuberculin skin test (TST) [1]. Unlike the TST, the ELISPOT assay does not seem to be susceptible to false-negative results for patients with disseminated tuberculosis [1], and it maintains its high sensitivity for HIV-infected patients with tuberculosis [3]. In addition, the ELISPOT assay is not confounded by prior BCG vaccination, as evidenced by uniformly negative results for BCG-vaccinated people with no history of tuberculosis exposure [1, 4, 5]; its specificity in these populations is 100%. The ELISPOT assay may thus prove clinically useful in the diagnostic assessment of patients with suspected active tuberculosis in regions where there is a low prevalence of latent tuberculosis infection.

The T cells enumerated by the ex vivo ELISPOT assay are effector cells that have recently encountered antigen in vivo and can rapidly release IFN-γ when reexposed to antigen [6]. In contrast, long-lived memory T cells, which persist long after clearance of the pathogen, are relatively quiescent and less likely to release IFN-γ during the short period of exposure to antigen in the ex vivo ELISPOT assay [6]. In a given individual, the frequency of effector T cells is thought to be largely driven by the antigen load, which is closely related to the bacterial load [6–8]. This is probably why the frequency of ESAT-6 peptide–specific T cells in patients with tuberculosis decreases progressively with successful antituberculous therapy, with an observed rate of decay of 5% (95% CI, 2.4%–8.4%) per week [9]. Thus, unlike the TST and serological tests, the ex vivo ELISPOT assay is dynamic, because it enumerates effector T cells, and the frequency of these cells reflects bacterial burden in vivo. For several chronic viral infections, there are blood tests that directly quantify virus load; in contrast, there is no such quantitative assay for determination of the bacterial load for tuberculosis. Quantitation of *M. tuberculosis*–specific T cells using the ELISPOT assay might, in theory, serve as an indirect measure of bacterial burden that could be used to monitor the response to tuberculosis treatment. However, individuals naturally differ in the level of T cell response that they mount for a given antigen load in vivo [6–8]. Therefore, although differences in T cell frequency within an individual over time reflect changes in bacterial burden, T cell frequencies cannot be used to compare bacterial load between individuals.

In this issue of *Clinical Infectious Diseases,* a report by Carrara et al. [10] describes the results of an investigation of whether a related ELISPOT assay can be

Received 18 November 2003; accepted 18 November 2003; electronically published 17 February 2004.

Financial support: A.L.'s research programme is funded by the Wellcome Trust. A.L. is a Wellcome Senior Clinical Research Fellow.

A.L. is a named inventor on patents relating to T cell–based diagnosis filed by the University of Oxford. Regulatory approval and commercialization of ELISPOT is being undertaken by a spin-out company of the University of Oxford (Oxford Immunotec Ltd.), in which A.L. has a share of equity and to which he acts as a scientific advisor.

Reprints or correspondence: Dr. Ajit Lalvani, Nuffield Dept. of Clinical Medicine, University of Oxford, Level 7, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom (ajit.lalvani@ndm.ox.ac.uk).

Clinical Infectious Diseases 2004;38:757–9

1058-4838/2004/3805-0022$15.00

used as a surrogate marker of bacterial burden to monitor the response to tuberculosis treatment. Using 2 immunogenic ESAT-6–derived peptides that span 38% of the ESAT-6 molecule instead of the full complement of peptides that span the whole molecule, the authors recently demonstrated that 20 (74%) of 27 patients with culture-confirmed tuberculosis had T cells that were responsive to these peptides in the ELISPOT assay [11]. For 18 of the patients who initially had positive results of the ELISPOT assay, Carrara et al. [10] performed a second ELISPOT assay after 3 months of antituberculous therapy and correlated changes in the frequency of ESAT-6 peptide–specific T cells with clinical, radiological, and microbiological response to treatment [10]. Strikingly, for all 13 patients who had a good response to therapy and whose culture results were negative for *M. tuberculosis* at 3 months, the frequency of ESAT-6 peptide–specific T cells had decreased to levels below the threshold for a positive result—that is, the ELISPOT assay result had turned negative. In contrast, the 5 patients with the least improvement after 3 months of treatment continued to have positive ELISPOT assay results, although these responses had decreased for 4 of them. For all 5 patients, *M. tuberculosis* could still be cultured from sputum, blood, or pleural fluid samples at this time point. These 5 patients apparently had the most extensive disease and poorest nutritional state before starting therapy. It is interesting to note that, after 6 months of treatment, clinical disease had resolved and clinical specimens had turned culture negative for these 5 patients, and, at this time point, ELISPOT assay results were negative. In contrast to what was observed for ESAT-6–derived peptides, ELISPOT assay responses to PPD did not correlate with response to therapy.

Thus, ELISPOT assay responses over time had a clear-cut correlation with clinical and microbiological response to therapy. ELISPOT assay results for the patients with a good response to treatment turned negative earlier than was observed in previous studies [9], but this may be related to the fact that initial ELISPOT assay responses in the patients in the study by Carrara et al. [10] were rather low. The relatively small number of patients, together with the paucity of clinical information provided, suggests that we should view these exciting new findings as promising preliminary data rather than as definitive proof. Moreover, it would have helped to see the individual patient data used to determine whether there had been a response to treatment at 3 months. Nonetheless, the report by Carrara et al. [10] is a good example of what can be learned by applying recent scientific advances at the bedside together with careful clinical observation. Their results suggest that the quantitative relationship between levels of effector T cells, antigen load, and bacterial burden can be exploited to monitor response to tuberculosis treatment. So what are the clinical implications of this?

First, the ELISPOT assay may prove to be useful for monitoring the efficacy of antituberculous therapy, as suggested by Carrara et al. [10]. However, there are, of course, several simple clinical, radiological, and microbiological parameters that we use to monitor response to tuberculosis treatment, and no single test can, or should, replace these. Rather, the ELISPOT assay may help as a useful adjunctive test, alongside comprehensive clinical evaluation. Second, there is an urgent need for improved antituberculous agents that allow shorter treatment courses. If the decrease in ELISPOT assay responses reflects decreasing bacterial burden during effective treatment, then the ELISPOT assay could enhance the evaluation of new therapies in clinical trials. This might prove to be especially useful for new pharmacological or immunological interventions for treating multidrug-resistant (MDR) tuberculosis, for which the response to treatment and the decline in bacterial burden are much slower and harder to predict. It is interesting that, in 2 patients with MDR tuberculosis for whom I tracked ELISPOT assay responses during treatment, the rate of decline was slower than it was in patients with drug-susceptible tuberculosis (unpublished observations).

What additional studies are needed to build on the interesting findings reported in this issue of *Clinical Infectious Diseases?* First, a larger prospective study that includes patients with MDR tuberculosis and uses predefined criteria for response to therapy is required. Second, it would be of interest to determine whether patients whose ex vivo ELISPOT assay results have turned negative after successful therapy still have detectable long-lived memory T cells in their blood. This might enable us, for the first time, to distinguish people whose *M. tuberculosis* infection has been fully treated and has cleared, leaving them with only memory T cells but no effector cells, from those who still harbor viable bacilli secreting proteins that provoke ongoing antigenic stimulation in vivo, leaving them with detectable numbers of effector T cells. This could help in evaluating patients who present with suspected recurrence of tuberculosis after chemotherapy. Finally, a major clinical challenge is the monitoring of preventive treatment of latent tuberculosis infection, for which there are there are no clinical, microbiological, or radiological parameters for assessing response to therapy. Given that the ELISPOT assay appears to be the most sensitive and specific new tool for detection of latent tuberculosis infection [5, 12, 13], a prospective study to investigate the effect of preventive therapy on ELISPOT assay responses over time would be helpful. A decline in response could make it possible for clinicians to monitor the effect of preventive therapy on latent infection.

References

1. Lalvani A, Pathan AA, McShane H, 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.
2. Lalvani A, Hill AV. Cytotoxic T-lymphocytes against malaria and tuberculosis: from natural immunity to vaccine design. Clin Sci (Lond) **1998**; 95:531–8.
3. Chapman AL, Munkanta M, Wilkinson KA, et al. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of *Mycobacterium tuberculosis*–specific T cells. AIDS **2002**; 16: 2285–93.
4. Lalvani A, Nagvenkar P, Udwadia Z, et al. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent *Mycobacterium tuberculosis* infection in healthy urban Indians. J Infect Dis **2001**; 183:469–77.
5. Lalvani A, Pathan AA, Durkan H, et al. Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. Lancet **2001**; 357:2017–21.
6. Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. Nat Rev Immunol **2002**; 2:251–62.
7. Klenerman P, Cerundolo V, Dunbar PR. Tracking T cells with tetramers: new tales from new tools. Nat Rev Immunol **2002**; 2:263–72.
8. Seder RA, Ahmed R. Similarities and differences in $CD4^+$ and $CD8^+$ effector and memory T cell generation. Nat Immunol **2003**; 4: 835–42.
9. Pathan AA, Wilkinson KA, Klenerman P, et al. Direct ex vivo analysis of antigen-specific IFN-γ–secreting CD4 T cells in *Mycobacterium tuberculosis*–infected individuals: associations with clinical disease state and effect of treatment. J Immunol **2001**; 167:5217–25.
10. Carrara S, Vincenti D, Petrosillo N, Amicosante M, Girardi E, Goletti D. Use of a T cell–based assay for monitoring efficacy of antituberculosis therapy. Clin Infect Dis **2004**; 38:754–6 (in this issue).
11. Vincenti D, Carrara S, De Mori P, et al. Identification of early secretory antigen target–6 epitopes for the immunodiagnosis of active tuberculosis. Mol Med **2003**; 9:105–11.
12. Ewer K, Deeks J, Alvarez L, et al. Comparison of T-cell–based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. Lancet **2003**; 361:1168–73.
13. Lalvani A. Spotting latent infection: the path to better tuberculosis control. Thorax **2003**; 58:916–8.