Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells

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Summary

Background Identification of individuals latently infected with *Mycobacterium tuberculosis* is an important part of tuberculosis control. The current method, the tuberculin skin test (TST), has poor specificity because of the antigenic cross-reactivity of purified protein derivative (PPD) with *M bovis* BCG vaccine and environmental mycobacteria. ESAT-6 is a secreted antigen that is highly specific for *M tuberculosis* complex, but is absent from *M bovis* BCG. With an enzyme-linked immunospot (ELISPOT) assay for interferon gamma, we have identified ESAT-6-specific T cells as an accurate marker of *M tuberculosis* infection.

Methods We did a prospective, masked study of 50 healthy contacts, with varying but well defined degrees of exposure to *M tuberculosis,* who attended an urban contact-tracing clinic. We assessed and compared the efficacy of our assay and TST for detection of symptomless infected individuals by correlation of test results with the degree of exposure to an infectious index case.

Findings The ESAT-6 ELISPOT assay results had a strong positive relation with increasing intensity of exposure (odds ratio=9·0 per unit increase in level of exposure [95% CI 2·6–31·6], p=0·001), whereas TST results had a weaker relation with exposure (1·9 [1·0–3·5], p=0·05). By contrast, ELISPOT results were not correlated with BCG vaccination status (p=0·7), whereas TST results were significantly more likely to be positive in BCG-vaccinated contacts (12·1 [1·3–115·7], p=0·03).

Interpretation This new antigen-specific T cell-based assay could allow more accurate identification of symptom-free individuals recently exposed to *M tuberculosis*, and thereby help to improve tuberculosis control.

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Introduction

Control of the global tuberculosis epidemic could be enhanced by identification and treatment of symptom-free people who are latently infected with *Mycobacterium tuberculosis*, as well as those with active disease. Even in countries with a low prevalence of tuberculosis, 30–40% of new cases are probably caused by recent transmission of *M tuberculosis* from infectious cases.[1] Immunocompetent individuals with *M tuberculosis* infection have a 10% risk of developing active disease in their lifetime; half this risk is in the first 1–2 years after exposure.[2] Chemoprophylaxis of recently infected individuals prevents the development of active tuberculosis,[3] and isoniazid preventive therapy is highly cost effective. It is therefore important to identify recently infected contacts and infected individuals at greatest risk of progression—eg, intravenous drug users, HIV-1 infected individuals, and children.[4,5]

The tuberculin skin test (TST) for diagnosis of latent *M tuberculosis* infection has remained almost unchanged for a century. Intradermal inoculation of purified protein derivative (PPD), which is a crude precipitate of more than 200 *M tuberculosis* antigens that are common to *M bovis* BCG and environmental mycobacteria, elicits a local cutaneous delayed-type-hypersensitivity response in sensitised individuals. The broad antigenic cross-reactivity of PPD causes the poor specificity of TST; a positive reaction can be a response not only to *M tuberculosis* infection, but also to BCG vaccination or exposure to environmental mycobacteria.[6–8] Because a third of the world's population is thought to be infected with *M tuberculosis*,[9] and most people have been vaccinated with BCG, accurate identification of those infected with *M tuberculosis* for targeted chemoprophylaxis is difficult.[5,6,8]

M tuberculosis infection evokes a strong cell-mediated immune response, and detection of T cells that are specific to this bacterium might be a means to detect infection. We therefore selected ESAT-6, a secreted antigen that is expressed in *M tuberculosis* complex[10] (*M tuberculosis, M bovis*, and *M africanum*), but is absent from all strains of *M bovis* BCG vaccine[11,12] and most environmental mycobacteria.[13] ESAT-6 is highly immunogenic in animal models of tuberculosis,[14,15] tuberculosis patients,[16–19] and people exposed to tuberculosis bacteria (contacts).[20,21] Cellular immune responses to ESAT-6 have been detected by standard in-vitro assays in 60–80% of tuberculosis patients.[22] We used a highly sensitive assay to detect ESAT-6-specific T cells, the ex-vivo ELISPOT assay for interferon gamma.[23] The ELISPOT assay is based on the principle of a sandwich-capture ELISA. The assay detects interferon-gamma molecules in the immediate vicinity of the T cell from which they were secreted, while still at a high concentration. Thus, each spot represents the footprint of an antigen-specific T cell that secretes interferon gamma (spot-forming cell). ELISPOT assay can detect antigen-specific T cells from blood without an in-vitro stimulation step,[23] hence incubation periods are short. We reported that ESAT-6-specific T cells were an accurate marker of *M tuberculosis* infection in 45 (96%) of 47 patients with culture-confirmed active disease.[21] We also noted that 22

(85%) of 26 TST-positive household contacts of patients with sputum smear-positive pulmonary tuberculosis had circulating ESAT-6-specific T cells.[21] These results suggested a new approach for the detection of recent tuberculosis infection.[24]

Our aim was to assess whether this approach could be used to identify individuals in routine clinical practice, who were at high risk of recent infection and to compare the results with those for TST. Unfortunately, no test can reliably confirm latent *M tuberculosis* infection in symptom-free individuals—ie, there is no gold standard for comparison—hence measurement of accuracy of a new assay is difficult. However, airborne transmission of *M tuberculosis* is promoted by close and prolonged contact with an infectious person,[25–27] and a key factor is the amount of time a contact spends sharing room air with an infectious individual.[28] We postulated that the ESAT-6 based ex-vivo ELISPOT assay would correlate better with intensity of exposure to *M tuberculosis* than TST, but would be independent of BCG-vaccination status. Therefore, we investigated people attending a contact-tracing clinic who had well defined but differing amounts of exposure to *M tuberculosis*.

Methods

Participants

We prospectively recruited healthy adult contacts from the Contact Tracing Clinic, Northwick Park Hospital, North West London Hospitals NHS Trust, London, UK. This clinic is held once a week and is attended by individuals thought to have been in contact with a newly identified case of tuberculosis. There were no exclusion criteria. We consecutively recruited 50 contacts of index cases who had sputum-smear-positive pulmonary tuberculosis. We identify cases in this report by number (HC 1–HC 54). No contacts refused consent. The population served by this clinic has a low HIV-1 seroprevalence. All participants underwent routine standardised TST (Heaf test), were questioned about past BCG vaccination, and examined for a BCG scar. We took chest radiographs of most contacts. All contacts were symptom-free (except HC 11). Chest radiographs were normal in 46 (96%) of 48 participants (abnormal in HC 11 and HC 6). 24 (48%) participants were of Asian or African origin. The others were white Europeans. Mean age was 33·8 years (SD 9·1), 35 were men, and 15 were women.

All contacts were interviewed by the project nurse, who recorded details of proximity to their index case and duration of contact. From these interviews, we assigned contacts to one of four predefined exposure categories. Category A contacts (close and prolonged exposure) had lived in the same household or shared their workplace office with their index case. Category B contacts (regular intermittent exposure) had been in the same room as their index case at least once per week for an estimated mean time of longer than 1 h (total) per week, for at least 4 weeks. Category C contacts (casual intermittent exposure) had been in the same room as their index case for an estimated mean time of less than 1 h (total) per week. Category D contacts had worked or studied in the same institution as an infectious person but had had no known contact with the index case or other tuberculosis patient.

Procedures

We took heparinised blood samples from all participants and transported them by courier to the laboratory in Oxford. All contacts underwent standard multiple puncture TST with a six-needle disposable head Heaf gun (Bignall Surgical Instruments, Littlehampton, UK) and concentrated 100 000 tuberculin units/mL PPD (Evans Medical, Liverpool, UK) in accordance with UK guidelines for assessment of tuberculosis contacts.[29] Heaf tests were done and read by two experienced chest-clinic nurses. Cutaneous induration was recorded 1 week later and was scored from grade 0–4 in accordance with standard guidelines. Grades 0 and 1 were scored as negative; grade 2 (equivalent to a Mantoux response of 5–14 mm induration after injection of 10 tuberculin units of PPD) as borderline; and grades 3 and 4 (equivalent to a Mantoux response of at least 15 mm induration) as positive. For an exposed contact, grades 3 or 4 are an indication for consideration of chemoprophylaxis.[29]

All index cases had pulmonary tuberculosis with one or more sputum samples that were positive for acid-fast bacilli by Ziehl-Neelsen stain and that grew *M tuberculosis* on culture. One index case did not produce a sputum sample, but three of three early-morning gastric-wash samples (containing swallowed bronchial secretions) were positive for acid-fast bacilli and grew *M tuberculosis*.

We amplified the *esat-6* gene from genomic DNA isolated from *M bovis* strain AN5 by PCR, and cloned it into plasmid vector pET21d (Novagen, Madison, WI, USA). *Escherichia coli* was transformed with this vector. Recombinant ESAT-6 was recovered from transformed *E coli* and purified by nickel-affinity chromatography. We did ex-vivo interferon-gamma ELISPOT assays, as described elsewhere, with 300 000 peripheral blood mononuclear cells per well.[21,23] We used purified recombinant ESAT-6 and PPD, in addition to negative controls (no added antigen) and positive controls (phytohaemagglutinin). Positive ESAT-6 test wells were defined as containing at least ten spot-forming cells more than, and at least twice as many as, negative control wells—a detection threshold of one per 30 000 peripheral blood mononuclear cells. Assays were done and independently scored by two scientists who were unaware of exposure category and TST results of contacts. ELISPOT assay wells containing more than 300 spot-forming cells cannot be counted accurately because the spots coalesce. 300 cells per well (equivalent to 1000 cells per million peripheral blood mononuclear cells) was therefore taken as the upper limit for accuracy.

Statistical analysis

We used multiple-logistic-regression models to simultaneously examine the relation between intensity of exposure and BCG-vaccination status with results of ESAT-6 ELISPOT, TST, and PPD ELISPOT. A regression model was fitted for each test. Each model estimated the trend between increasing intensity of exposure and the proportion of positive contacts as an odds ratio (multiplicative increase in odds of test result being positive per unit increase in exposure category). Furthermore, the models estimated the relative increase in odds of test results being positive in BCG-vaccinated contacts compared with unvaccinated contacts. Statistical significance was shown by $p<0·05$.

Results

20 participants were contacts of an index case (IC). Figure 1 shows the proximity of these contacts to IC, and results of ESAT-6 ELISPOT and TST. IC shared a house with two cohabitants, including a work colleague, and worked consecutively in two separate offices within a large university building. He had a productive cough throughout the last 3 months that he worked in office A (figure 1) and during the next 2 months when he worked in office B (figure 1), before his admission to hospital.