Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak

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Summary

Background The diagnosis of latent tuberculosis infection relies on the tuberculin skin test (TST), which has many drawbacks. However, to find out whether new tests are better than TST is difficult because of the lack of a gold standard test for latent infection. We developed and assessed a sensitive enzyme-linked immunospot (ELISPOT) assay to detect T cells specific for *Mycobacterium tuberculosis* antigens that are absent from *Mycobacterium bovis* BCG and most environmental mycobacteria. We postulated that if the ELISPOT is a more accurate test of latent infection than TST, it should correlate better with degree of exposure to *M tuberculosis*.

Methods A large tuberculosis outbreak in a UK school resulted from one infectious index case. We tested 535 students for *M tuberculosis* infection with TST and ELISPOT. We compared the correlation of these tests with degree of exposure to the index case and BCG vaccination.

Findings Although agreement between the tests was high (89% concordance, κ=0·72, p<0·0001), ELISPOT correlated significantly more closely with *M tuberculosis* exposure than did TST on the basis of measures of proximity (p=0·03) and duration of exposure (p=0·007) to the index case. TST was significantly more likely to be positive in BCG-vaccinated than in non-vaccinated students (p=0·002), whereas ELISPOT results were not associated with BCG vaccination (p=0·44).

Interpretation ELISPOT offers a more accurate approach than TST for identification of individuals who have latent tuberculosis infection and could improve tuberculosis control by more precise targeting of preventive treatment.

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Introduction

Identification and treatment of people who have latent tuberculosis infection by targeted tuberculin skin testing and preventive therapy is a cornerstone of tuberculosis control in developed countries.[1] The main drawback of the tuberculin skin test (TST) is poor specificity, since previous *Mycobacterium bovis* BCG vaccination and environmental mycobacterial exposure can lead to false-positive results.[2–4] More than half the burden of tuberculosis in developed countries is carried by foreign-born immigrants from high-prevalence countries, among whom BCG vaccination and environmental mycobacterial exposure are common.[5,6] The TST also has several operational drawbacks, including the need for a return visit and operator-dependent variability in placement and reading of the test. A more accurate rapid test for latent infection is a major priority for improved tuberculosis control.[7]

The identification of genes in the *M tuberculosis* genome that are absent from *M bovis* BCG[8] and most environmental mycobacteria[9] offers an opportunity to develop more specific tests for *M tuberculosis* infection.[10] Early secretory antigen target-6 (ESAT-6) and culture filtrate protein 10 (CFP10) are two such gene products that are strong targets of the cellular immune response in tuberculosis patients and contacts.[11,12] The presence of ESAT-6-specific T cells, detected by the rapid ex-vivo enzyme-linked immunospot (ELISPOT) assay for interferon-gamma,[13] is a highly sensitive and specific marker of *M tuberculosis* infection in patients who have culture-confirmed tuberculosis; its sensitivity is substantially higher than that for the TST.[14,15] In a UK pilot study of 50 contacts at risk of latent tuberculosis infection, we noted a correlation between ESAT-6 ELISPOT results and the extent of exposure to tuberculosis cases,[16] whereas unexposed people were uniformly ELISPOT-negative.[17,18]

In February, 2001, a secondary school student who had had a chronic cough for 9 months was diagnosed with sputum-smear-positive cavitatory pulmonary tuberculosis. The health authority screened 1128 of 1208 students at the school with TST and diagnosed 69 secondary cases of active tuberculosis and 254 cases of latent infection. This outbreak presented a unique opportunity to compare the effectiveness of the ELISPOT assay with the TST.

In the absence of a gold standard reference test, direct assessment of the sensitivity and specificity of a new test for latent tuberculosis infection is impossible.[4] However, since airborne transmission of *M tuberculosis* is promoted by increasing duration and proximity of contact with an infectious case,[19–21] a key determinant of infection is the amount of time spent sharing room air with the source case.[22,23] We formed the hypothesis that if the ELISPOT assay is a more sensitive and specific test than the TST, it should correlate more closely than the TST with degree of exposure to *M tuberculosis* and should be independent

of BCG vaccination status. Two measures of exposure were prespecified at the time of study design: proximity to the index case, based on school class and year, and hours of direct classroom contact. Three features of this outbreak made it particularly suitable for this investigation: there was one infectious index case with several hundred contacts; the outbreak occurred in an enclosed environment; and school timetables permitted precise quantification of the amount of time each child spent sharing room air with the source case.

Patients and methods

Participants

The study was approved by the Leicestershire research ethics committee. We invited 963 students, aged 11–15 years, from the same school as the index case to participate. We obtained written informed consent from 594 (62%) children and their parents. In May and June, 2001, the school nurses interviewed 550 (57% of the total invited) of these children about place of birth and history of tuberculosis exposure outside school. At the same time they drew 10 mL blood samples that were stored in sequentially numbered heparinised containers.

TST and ELISPOT testing

Leicestershire Health Authority screened 1128 children with the Heaf test, in accordance with UK guidelines for tuberculosis contacts (table 1),[24] 535 of whom were in our sample of 550. Screening was done over 2 weeks, from March 26 to April 11, 2001, 2 months after the index case was admitted to hospital for treatment.

Tuberculin skin testing was done by standard multiple-puncture Heaf test with a six-needle disposable-head Heaf gun (Bignall Surgical Instruments, Littlehampton, UK)[25] and concentrated purified protein derivative 100 000 tuberculin units per mL (Evans Medical, Liverpool, UK), in accordance with national guidelines.[24] Heaf tests were administered and read by the medical and nursing staff of the outbreak management team. Cutaneous induration was scored 1 week later, in accordance with standard guidelines, from grade 0 to 4.[25] Generally, although the read-out of the automated Heaf test is quantified less precisely than the Mantoux test—ie, grades 0–4 instead of mm of induration, a continuous variable—the two tests generally correlate well with each other.[24–26]

Students who reported symptoms underwent chest radiography and clinical assessment for possible active tuberculosis, irrespective of skin test results. Asymptomatic students with Heaf grades 0 or 1 or Heaf grade 2 and a BCG scar or documented history of BCG vaccination were deemed uninfected[24,25] and no action was taken; students with Heaf grades 3 or 4 (irrespective of BCG vaccination history) or grade 2 with no evidence of previous BCG vaccination were deemed infected.[24,25] All underwent chest radiography and those with normal radiographs were deemed to have latent tuberculosis infection and received 3 months' chemoprophylaxis with rifampicin and isoniazid. Students with abnormal radiographic findings or with symptoms were further assessed in hospital for active tuberculosis; those with positive cultures for *M tuberculosis* from clinical samples or positive radiological or clinical findings suggestive of tuberculosis were classified as having active tuberculosis disease. These students were treated with standard short-course chemotherapy for 6 months, including pyrazinamide and ethambutol for the first 8 weeks.

We did ELISPOT assays in Oxford on blood samples from 545 of the 550 students, 2–4 h after venepuncture. Samples were processed and scored by two scientists who had no access to personal identifiers or TST results. How ELISPOT assays are done has been previously described;[13,14] for this study we used a simplified, faster protocol incorporating ELISPOT plates precoated with monoclonal antibody to interferon-gamma (Mabtech AB, Stockholm, Sweden), and a detector monoclonal antibody to interferon-gamma preconjugated to alkaline-phosphatase (Mabtech). Plates were seeded with $2{\cdot}5\times10^5$ peripheral blood mononuclear cells per well: duplicate wells contained no antigen (negative control), phytohaemagglutinin (positive control; ICN Biomedicals, OH, USA), recombinant dimeric ESAT-6 (dimESAT-6), or one of 12 different peptide pools derived from ESAT-6 and CFP10. After overnight incubation at 37°C, 5% carbon dioxide$_2$, plates were developed with preconjugated detector antibody and chromogenic substrate, BCIP/NBT^{PLUS} (Moss Inc, Pasadena, MD, USA).

Characteristics	
Demographic	
Mean (range) age (years)	13·1 (11–15)
Sex (male/female)	267/268
School year	
7	152 (28·4%)
8	132 (24·9%)
9	148 (27·7%)
10	103 (19·8%)
Risk factors for tuberculosis exposure outside school	
History of household contact	36 (6·7%)
Born in high-prevalence country*	76 (14·2%)
Ethnic origin	
Indian	436 (81·5%)
Pakistani	35 (6·5%)
White	19 (3·6%)
Mixed race	19 (3·6%)
Black African	12 (2·2%)
Black Caribbean	7 (1·3%)
Other	7 (1·3%)
Clinical	
BCG vaccinated	467 (87·3%)
Heaf grade (equivalent induration after 10 TU Mantoux)	
Negative	
0 (<5 mm)	141 (26·4%)
1 (<5 mm)	129 (24·1%)
2 (5–14 mm) and BCG vaccinated	110 (20·6%)
Positive	
2 (5–14 mm) and BCG unvaccinated	10 (1·9%)
3 (>15 mm)	83 (15·5%)
4 (>15 mm)	62 (11·5%)
Diagnosis based on conventional criteria	
Latent tuberculosis infection	128 (23·9%)
Active tuberculosis disease	27 (5·0%)

*India (44), Pakistan (nine), Bangladesh (three), Africa (Malawi, Kenya, and Tanzania, 14), Portugal (two), and Greece, Malaysia, Sri Lanka, and Turkey (one each).

Table 1: **Characteristics of students tested by TST and ELISPOT**

Assays were scored by automated ELISPOT counter (AID-GmbH, Strassberg, Germany). We scored test wells as positive if they contained a mean of at least five more spot-forming cells than the mean of the negative control wells and this number was at least twice the mean of the negative control wells. This cut-off[14] was predefined before the results were revealed. Assays were deemed positive if there was a positive response to one or more pools of the ESAT-6-derived or CFP10-derived peptides, or to dimESAT-6.

As previously described,[14] we used peptides spanning the length of ESAT-6 and CFP10 (ResGen, Huntsville, AL, USA). Each peptide was 15 aminoacids long and overlapped its adjacent peptide by 10 residues; purity was more than 70%. Peptides were arranged into 12 pools comprising two arrays of six pools each, where each array contained all 35 peptides from both molecules in

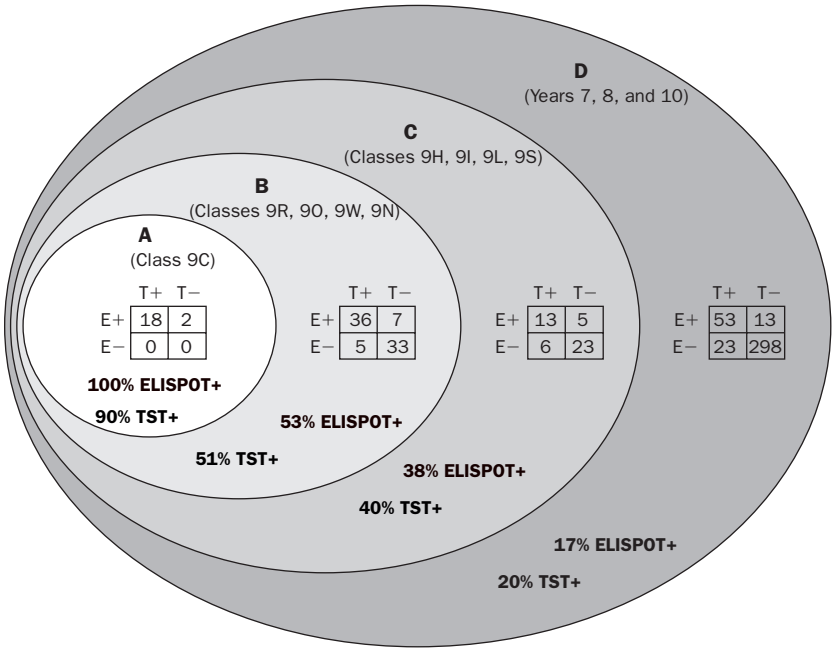

Figure 1: **TST and ELISPOT results for students stratified by decreasing proximity to index case based on school year and class**

T+=TST positive. T−=TST negative. E+=ELISPOT positive. ELISPOT−=ELISPOT negative.
A: students in same class as index case. B: students in classes in same year who regularly shared lessons with index case. C: students in the four remaining classes in same year who shared only weekly school events but no lessons with index case. D: students in different years who shared no school events with index case.

contrasting combinations, so that each peptide was tested in quadruplicate.

We cloned, expressed, and purified DimESAT-6 from culture supernatant of recombinant *Lactococcus lactis*; purity was more than 95%.

Ascertainment of exposure

We classified school students into four groups of decreasing degrees of exposure to the index case, based on proximity and shared activities in school: the same class as the index case; students in classes in the same year (year 9) who regularly shared classes with the index case; students in classes in the same year who shared only weekly events with the index case; and students in different years (7, 8, and 10) who shared no school events with the index case (figure 1). For students in the same school year, we used the school timetable to quantify direct exposure to the index case, taking into account the attendance record of the index case during the likely infectious period, which, on the basis of duration of cough and associated symptoms, was 9 months. Since the index case mixed with different students for each academic subject, substantial numbers of students were exposed. We classified students from other school years (years 7, 8, and 10) who had lessons in classrooms immediately after they had been vacated by the index case as indirectly exposed. Direct and indirect exposure are expressed in school-weeks (equivalent to 26 h 40 min).

Statistical methods

We focused the analysis on estimating the strength of association between degree of exposure to the index case and the ELISPOT and TST test results. Odds ratios are a function of test sensitivity and specificity,[27] and increase as one or both of these measures increase. For latent tuberculosis infection we could not estimate test sensitivity and test specificity directly, but were able to estimate odds ratios relating results of each test to the likelihood of infection. We estimated the increase in odds of a positive test result for unit increase in exposure by logistic regression. We used matched-pairs logistic regression to assess the significance of the difference in the associations between the tests, taking account of the correlation between TST and ELISPOT test results. Similar analyses were undertaken to investigate whether ELISPOT and TST test results were associated with BCG vaccination, place of birth, and household tuberculosis contact. All reported p values are two sided. We investigated trends with use of the χ^2 statistic. Comparisons between proportions were derived with Fisher's exact test. We did all analyses with STATA (version 7.0).

Role of the funding source

The sponsors of the study had no role in the study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit the paper for publication.

Results

ELISPOT and TST results were available for 535 students—44·3% of the school. Our sample was representative in terms of the proportion of non-white children (97% in our sample *vs* 93% in the whole school); UK-born children (86 *vs* 86%); children diagnosed with active tuberculosis (5 *vs* 6%); and participants deemed to have latent tuberculosis infection on the basis of TST result (24 *vs* 23%, table 1).

The odds of a test result being positive for each increase across the four stratified exposure groups increased by a factor of 2·78 (95% CI 2·22–3·48,

	ELISPOT	p	TST*	p	p for TST *vs* ELISPOT
Exposure to *M tuberculosis* in school					
Stratified exposure groups (whole school, n=535)	2·78 (2·22–3·48)	<0·0001	2·33 (1·88–2·88)	<0·0001	0·03
Direct exposure (weeks) in year 9 (n=148)	2·51 (1·58–3·99)	<0·0001	1·30 (1·10–1·54)	0·002	0·007
Indirect exposure (weeks) in years 7, 8, and 10 (n=387)	1·87 (0·87–4·04)	0·11	1·63 (0·78–3·43)	0·20	0·69
Risk factors for exposure to *M tuberculosis* outside school					
History of household tuberculosis contact (n=36)	1·99 (0·99–3·97)	0·05	2·34 (1·18–4·63)	0·02	0·61
Born in high-prevalence country (n=76)	1·17 (0·69–1·99)	0·56	1·74 (1·05–2·88)	0·03	0·09

*Positive result defined as Heaf grade >2 or heaf grade 2 without BCG.

Table 2: **Odds ratios (95% CI) of the relations of ELISPOT and TST with intensity of *M tuberculosis* exposure in school and with risk factors for exposure outside school**

	Vaccinated (n=467)	Unvaccinated (n=68)	p for vaccinated *vs* unvaccinated
ELISPOT			
Positive	131 (28·1%)	16 (23·5%)	0·44
Negative	336 (71·9%)	52 (76·5%)	
Heaf grade			
4	52 (11·1%)	10 (14·7%)	
3	81 (17·6%)	2 (2·9%)	
2	110 (23·6%)	10 (14·7%)	0·002*
1	116 (24·8%)	13 (19·1%)	
0	108 (23·1%)	33 (48·5%)	

*χ^2 test for trend across all five Heaf grades.

Table 3: **Effect of previous BCG vaccination on ELISPOT and TST results**

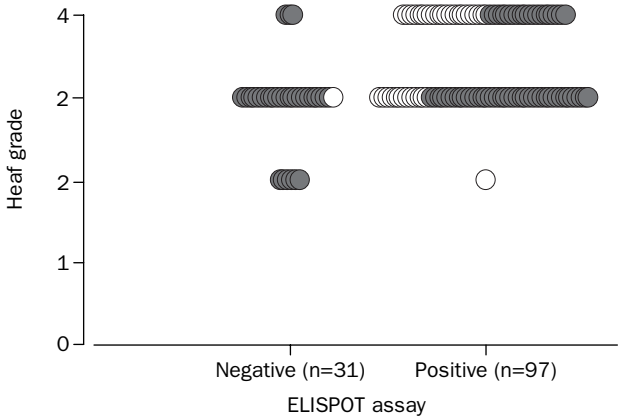

Figure 2: **Stratification of TST-positive students with presumed latent tuberculosis infection by ELISPOT result**
128 TST-positive students with no clinical or radiographical signs of active tuberculosis disease. Each circle represents one student: white=students with direct classroom exposure to index case; grey=students with no direct exposure.

p<0·0001) for the ELISPOT assay and 2·33 (1·88–2·88, p<0·0001) for the TST. The ELISPOT assay correlated significantly better with increasing exposure across the four groups than did the TST (p=0·03; figure 1, table 2).

Direct exposure of the 148 children in year 9 ranged from 0 to 17 school weeks: 57 students had some direct classroom exposure, with a median of 2·2 school weeks (IQR 1·4–13·4). The odds of a positive ELISPOT result increased by a factor of 2·51 (1·58–3·99, p<0·0001) with each week of direct exposure, which was significantly higher (p=0·007) than that for the TST (odds ratio 1·30 [95% CI 1·10–1·54], p=0·002; table 2).

Of the 387 children in years 7, 8, and 10, 196 pupils had indirect exposure, up to a maximum of 1·16 weeks. Although ELISPOT and TST were more likely to be positive with increasing exposure, neither showed a significant correlation (table 2).

ELISPOT assay and TST were positively correlated with a history of household tuberculosis contact (n=36, table 2). 76 children were born in countries with a high prevalence of tuberculosis and climates associated with increased exposure to environmental mycobacteria (table 1). The mean duration of residence in these countries was 7·8 years. TST results, but not ELISPOT results, were significantly associated with birth in one of these regions (table 2).

For 362 students, the date of BCG vaccination was documented in the Leicestershire Health Authority records, of whom 323 were vaccinated at birth. An additional 105 students had BCG scars but the date of vaccination was not available because they were born outside Leicester; 101 were born in countries where BCG vaccination is given at birth. Therefore 424 (91%) of 467 BCG-vaccinated students were vaccinated at birth. The ELISPOT assay showed no significant relation with BCG vaccination status (p=0·44, table 3). By contrast, BCG-vaccinated children were significantly more likely to have higher Heaf grades than unvaccinated children (p=0·002), with substantially more Heaf grade 3 (81 of 467 *vs* 2 of 68, p=0·001), and grade 2 results in BCG-vaccinated individuals (table 3).

Of the 128 participants presumed to have latent tuberculosis infection on the basis of a positive TST with no evidence of active tuberculosis, 97 (76%) tested positive with ELISPOT. This ELISPOT-positive subgroup had significantly higher Heaf grades and significantly more exposure to *M tuberculosis* than did the ELISPOT-negative students. Heaf grades were significantly higher among ELISPOT-positive students than among ELISPOT-negative students (p<0·0001, figure 2). In the ELISPOT-positive group there were significantly more students with direct exposure to the index case than in the ELISPOT-negative group (35 of 97 *vs* one of 31, p<0·0001; figure 2).

Agreement between TST and ELISPOT was high (κ=0·72 [95% CI 0·64–0·80], p<0·0001), with concordant results in 475 (89%) students (table 4). For students in whom test results were discordant, it is impossible to know for certain which test was correct because there is no reference test. However, table 4 shows that an isolated positive ELISPOT result (ie, one associated with a negative TST) was a strong predictor of *M tuberculosis* exposure, whereas an isolated positive TST result was not. This finding suggests that isolated positive ELISPOT results are more likely to be true positives than are isolated positive TST results. For students with positive TST and ELISPOT results, the relative risk of direct exposure to the index case, compared with that for students with negative TST and ELISPOT, was 17·6

	Students' exposure to *M tuberculosis*			Mean (range) duration of direct exposure (weeks)	Possible causes of false-positive results	
	Direct exposure (n [%])	In index case's class (n [%])	In year 9 (n [%])		BCG vaccinated (n [%])	Foreign born (n [%])
Test results						
TST+ ELISPOT+ (n=121)	42 (34·7)	18 (14·9)	67 (55·4)	2·42 (0–16·9)	106 (87·6)	19 (15·7)
TST– ELISPOT+ (n=26)	6 (23·1)	2 (7·7)	15 (57·7)	1·22 (0–16·2)	25 (96·2)	4 (15·4)
TST+ ELISPOT– (n=34)	2 (5·9)	0	11 (32·4)	0·12 (0–2·8)	27 (79·4)	11 (32·4)
TST– ELISPOT– (n=354)	7 (2·0)	0	55 (15·5)	0·03 (0–2·21)	309 (87·3)	42 (11·9)
p						
TST–/ELISPOT+ *vs* TST– ELISPOT–*	<0·0001	0·005	<0·0001	NT	0·34	0·54
TST+/ELISPOT– *vs* TST– ELISPOT–*	0·18	1·0	0·03	NT	0·19	0·003

NT=no statistical test undertaken due to skewed distributional shape because most students had no direct exposure. *p values are for comparison of epidemiological characteristics of students with an isolated positive test result *vs* characteristics of students negative for both tests.

Table 4: **Analysis of concordant and discordant test results**

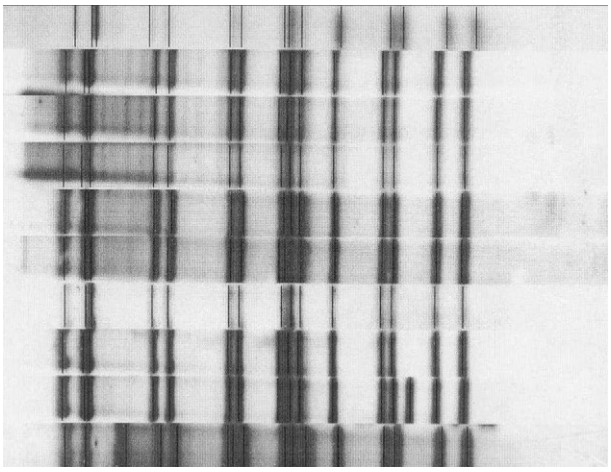

Figure 3: **Restriction fragment length polymorphism patterns of *M tuberculosis* isolates from students at school**
Top row is isolate from index case; other nine rows are isolates from secondary cases from who *M tuberculosis* was cultured.

(95% CI 8·1–38·0, p<0·001); for those with negative TST and positive ELISPOT results it was 11·7 (4·2–33·2, p<0·001); and for those with postive TST and negative ELISPOT results, it was 2·97 (0·6–13·7, p=0·18).

Molecular strain typing by variable-number tandem repeat, mycobacterial interspersed repetitive unit, and spoligotyping showed that all nine secondary isolates of *M tuberculosis* from students at the school were identical to that of the index case. IS6110-based restriction fragment length polymorphism (figure 3) showed that seven of the nine secondary isolates were identical to that of the index case, whereas two were very similiar, differing by one and three bands each.

Discussion

In the absence of a gold standard test for latent tuberculosis infection, the sensitivity and specificity of the ELISPOT assay or the TST cannot be directly quantified.[4] However, given that the likelihood of latent tuberculosis infection is determined by exposure to *M tuberculosis*,[19–23] we were able to rank the tests according to their diagnostic accuracy. Agreement between TST and ELISPOT results was high, but discordance in 11% of students shows that the tests are not equivalent. Our results indicate that ELISPOT probably has higher sensitivity and specificity than TST. First, the significantly closer correlation of ELISPOT than TST with degree of exposure to *M tuberculsosis* suggests a higher sensitivity for detection of latent infection. Second, TST, but not ELISPOT, was confounded by BCG vaccination, despite 11–15 years having elapsed since vaccination, which suggests a higher specificity for the ELISPOT assay.

TST and ELISPOT were more likely to be positive in students who had a history of household tuberculosis contact, a marker of *M tuberculosis* exposure outside school, than in students without such a history. By contrast, for the students born in high-prevalence countries, mainly Africa and Asia (a risk factor for environmental myobacterial exposure[3,4] and *M tuberculosis* exposure) only the TST was significantly more likely to be positive. Given that the ELISPOT assay correlates strongly with all other measures of *M tuberculosis* exposure, its independence from foreign birth suggests that, unlike TST, it is not confounded by environmental myobacterial exposure.

The high specificity of ELISPOT might explain the strong relation between positive ELISPOT results and TST induration size in individuals who have positive TST results. The size of the TST response is positively associated with higher tuberculosis case rates during follow-up; thus, the ELISPOT assay may have identified the subgroup of TST-positive individuals who actually have latent tuberculosis infection. These individuals are distinct from those whose weakly positive TST responses represent false-positive results due to antigenic cross reactivity of purified protein derivative. Moreover, the ELISPOT-positive group had substantially more exposure to *M tuberculosis* than did the ELISPOT-negative group. This improved specificity of the ELISPOT could help to avoid unnecessary chemoprophylaxis in uninfected individuals; this ability to screen out false-positive TST results will become increasingly important as the prevalence of latent infection falls in low-prevalence countries.[1] The cross-reactivity of purified protein derivative may explain why a new whole-blood interferon-gamma ELISA based on purified protein derivative seemed to be confounded by BCG.[28,29]

There is compelling evidence that the outbreak we studied was due to one index case, who was the first symptomatic case of pulmonary tuberculosis in the school.[30] The molecular epidemiology also suggests that this was a point-source outbreak. Only two other children were potentially infectious.[30] Both children were symptomatic for less than 2 weeks before admission to hospital and both were in year 11, which did not participate in the study. Moreover, their *M tuberculosis* isolates were identical to that of the index case by all four typing methods.

The high rates of tuberculosis infection and disease at the school are unlikely to merely reflect the epidemiology of tuberculosis in the local community. First, this outbreak accounted for a third of all tuberculosis cases in Leicester in 2001. Second, all 1226 household contacts of the 69 tuberculosis cases and 254 cases of latent tuberculosis infection were screened by the health authority and no cases of infectious pulmonary tuberculosis were identified. Third, four other Leicester schools were screened by TST, and the rates of positive skin tests were 1–4%. Fourth, when year 8 students at this school underwent Heaf testing in 1997–98 only 2·7% were positive.

The minimum exposure to an infectious person that is required for *M tuberculosis* transmission is unknown, but must be low, since many well-documented cases of infection result from brief exposure[31] and many students who did not share lessons with the source case must have acquired infection in this way. The amount of exposure required before transmission of *M tuberculosis* becomes inevitable is also unknown. Since all students with 5 or more school-weeks of exposure had positive results on the ELISPOT assay, however, our findings suggest that 130 h sharing room air with a person with sputum smear-positive cavitatory tuberculosis is certain to result in infection.

Longitudinal assessment of the positive predictive value of this assay for subsequent development of active tuberculosis will be necessary. In one report workers suggest that T-cell responses to ESAT-6 in healthy contacts are associated with subsequent active disease.[32] Students in our study who had positive ELISPOT but negative TST results, who have not had chemoprophylaxis, are receiving close clinical and radiographic follow-up.

We used the Heaf test, because it is used for tuberculin testing in contact investigations in the UK, and is stipulated in national guidelines.[24] Since the Mantoux

method is more widely used internationally, ELISPOT should be compared in the future with this method; we have recently started such studies in several countries.

ELISPOT gives quantitative results the morning after taking a blood sample and is more convenient, objective, and rapid than the TST. Although TST is cheap, related indirect costs are associated with return visits and the trained staff required to administer and read the test. Introduction of ELISPOT might initially increase the cost of tuberculosis control, but the savings that would follow from improved diagnosis of latent tuberculosis infection could make it very cost effective in the long term. Better detection of latent infection would lessen the number of cases of active tuberculosis and, therefore, the attendant cost of diagnosis, hospital admission and contact tracing. Fewer false-positive results in uninfected contacts would avoid the costs associated with unnecessary chemoprophylaxis and its associated toxic effects.

Contributors

Ajit Lalvani, Katie Ewer, Jonathan Deeks, Gerry Bryant, and Philip Monk designed the study. Ajit Lalvani coordinated the study. Katie Ewer and Lydia Alvarez did the ELISPOT assays. Sue Waller interviewed and enrolled all the participants. Demographic information was obtained and recorded by Sue Waller, Gerry Bryant, and Philip Monk. Jonathan Deeks computed the duration of exposure and did the statistical analysis. Peter Andersen synthesised the recombinant ESAT-6 and provided technical advice and support. Ajit Lalvani wrote the paper with help from Katie Ewer and Jonathan Deeks, and all researchers reviewed the final report.

Conflict of interest statement

AL is the named inventor on several patents related to T-cell-based diagnosis filed by the University of Oxford since 1996. Regulatory approval and commercialisation of the ELISPOT assay will be undertaken by a spin-out company of the University of Oxford (Oxford Immunotec), in which AL has an equity stake. PA is the named inventor of several patents filed by Statens Serum Institute relating to the discovery of *M tuberculosis*-specific antigens.

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